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MORPHOLOGY AND PHYSIOLOGY OF THE AXIS IN CUCURBITACEAE[1]

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(WITH PLATES I–III AND FIVE FIGURES)

Introduction

From the time of HARTIG, a copious literature has developed on the origin and history of the internal phloem. The writer investigated the embryology and developmental history of *Cucumis Melo* var. *flexuosus* Naud., in which he showed that the internal phloem arises by continuation inward from the normal intrafascicular cambium of cells that become cambioid, and that eventually surround the xylem. This zone produces, on its inner face adjoining the pith, the first rudiments of an internal phloem. Through continued cambial activity, the masses of internal phloem are gradually enlarged to form the well known bicollateral bundles of the Cucurbitaceae. This internal phloem maintains its position throughout. Observation of certain interesting details during the progress of that work induced the writer to engage in the study of the more advanced conditions in the root, hypocotyl, and gradually maturing stem of this and other genera of the Cucurbitaceae.

The presence of internal phloem in stems was first discovered and described by HARTIG (**6**) in *Cucurbita Pepo* in 1854. VON MOHL (**23**) announced its presence, the year following, in

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Apocynaceae, Solanaceae, and Compositae. SCHREIBER (15) in 1865 announced its presence in Lythraceae, and RUSSOW (13) in a great many diverse families. In addition, VESQUE (21), PETERSEN (9), and SOLEREDER (16, 17) give us a fair knowledge of the extent of this feature in other families of the dicotyledons. As given by SOLEREDER, they include Vochysiaceae, Melastomaceae, Myrtaceae, Lythraceae, Onagraceae, Cucurbitaceae, Asclepiadaceae, Apocynaceae, Solanaceae, Gentianaceae, and Loganiaceae, as well as in most Convolvulaceae and in some genera of the Acanthaceae, Thymelaeaceae, Penaeaceae, Euphorbiaceae, Polygonaceae, and Campanulaceae. It will be noticed that this advanced developmental condition is rare in the primitive dicotyledons or Incompletae (Apetalae); is more frequent in the Apopetalae (Polypetalae); and most frequent, as well as most perfectly evolved, in the Sympetalae (Gamopetalae).

DEBARY (2), referring to the leaf trace bundles in the Cucurbitaceae, states that phloem areas "are frequently connected by means of a narrow band, fringing the lateral edge of the bundle, and containing some sieve tubes, so that in these cases the bundle, strictly speaking, belongs to the concentric type." Further, referring to the leaf traces of the Melastomaceae and other families, he states that "in many of these the inner phloem is so widely separated from the rest of the bundle that it may best be regarded as a distinct strand of sieve tubes." DEBARY originated the term "bicollateral."

GERARD (4) traced the course of the bundles from the root to stem in *Cucumis Melo* and *Cucurbita maxima*, and found that the phloem of the root gave off strands, some of which were continuous with the internal phloem of the stem. He concluded that the internal phloem is merely a part of the external that gets placed on the inner face of the bundle.

PETERSEN (9) described the bicollateral bundle as agreeing with the collateral, with the addition of an internal phloem area.

WEISS (24) determined that all the bundles in the Cucurbitaceae were leaf trace bundles. In the petioles, at the point where the leaf veins branch off from the main bundles, the internal phloem unites with the external, and the bundles are no longer bicollateral.

FISCHER (3), in tracing the transition from hypocotyl to root, found that the internal phloem gradually died out and ended blindly below. He states that the internal phloem passes to the outside of the primary xylem and forms a ring between this and the secondary wood, disappearing lower down, but long remaining distinguishable in the medullary rays.

HERAIL (7) stated that the Cucurbitaceae alone possess a true bicollateral bundle, because the development of all three parts of the bundle is synchronous.

POTTER (11) observed that the mode of increase in thickness in the stem of *Cephalandra indica* is the same as that of ordinary dicotyledons. It differs in that its bundles are disposed in two circles rather than in one. He subscribes to the view of DEBARY that these two concentric rings of bundles behave as a single ring which bends alternately outward and inward. In a later paper (12) he calls attention to the fact that while climbing cucurbits possess a sclerenchymatous ring of cortical tissue, the shrubby members of the family, sufficiently supported by a relatively greater amount of xylem, do not possess this feature. Further, in the shrubby forms, the need for additional phloem is met by the formation of metaphloem by the intrafascicular cambium, and thus the amount of internal phloem is not large.

LAMOUNETTE (8) found that the external phloem had developed extensively in the region between the "heel" and the first rootlets of the hypocotyl, while the pith parenchyma was giving rise to an internal phloem. No communication was observed between the two. The formation of the internal phloem was thus subsequent to that of the external. LAMOUNETTE concluded that the internal phloem development is abnormal, and that it is due to the activity of certain pith cells. He advocated the abandonment of the term "bicollateral" in view of this method of origin.

SCOTT and BREBNER (14) indicate the connection of the internal and external phloem areas. "In the transitional regions the strands of internal phloem successively pass out between the converging xylem bundles and one by one reach the strands of the external phloem, with which they fuse." The writer does not subscribe to this centrifugal behavior of the internal phloem. The authors

suggest that the internal phloem is of advantage in that it is phloem sheltered by the wood cylinder, that the tissues are more concentrated, and that communication with the pith is more easily established.

BARANETSKY (1) states that "the internal phloem bundles in the Cucurbitaceae, when provided with their own wood, represent, doubtless, independent vascular bundles." Their independence is demonstrated by their branching and by their passing from one normal bundle to another. He would regard the presence of internal phloem as an evolutionary development, and cites its presence in some of the Sympetalae in support of this idea.

PITARD (10) found in *Cucurbita Pepo* strands of phloem in the rays of the stem at the edge of the wood which are connected by branches with the internal phloem of the bundles.

TONDERA (20), investigating *Sicyos angulatus*, concluded that the internal phloem is derived without cambial action, and is placed directly in front of the protoxylem. He indicates the presence of seven outer and three inner bundles (instead of five outer and five inner), confounding two bundles which, as often happens, are of greatly reduced size as compared with the other three. He considers that the two smallest bundles, which he terms of the outer circle and which in reality are of the inner, consist of phloem without any xylem. His paper is concerned with tracing the relation and disposition of the vascular bundles in connection with the leaf supply.

VON FABER (22), studying *Cucurbita Pepo*, found that at the growing apex both external and internal cambiums appear on either side of the developing vascular bundle, before any visible differentiation of spiral or other wood elements can be traced. He favors the opinion of BARANETSKY (1) with regard to exotic Cucurbitaceae, where not only an internal cambium, but also an internal xylem arises on the inner face of the phloem and cambium (corresponding to the "obcollateral bundles" of the writer), so that an internal phloem represents a developing commencement of a complete bundle. He upholds the term "bicollateral," and regards the development of both phloem areas as being centripetal, that is, toward the protoxylem.

MISS THOMPSON (19), in a study of the anatomy of *Gelsemium sempervirens*, indicates the absence of any internal phloem in the

lower part of the hypocotyl and the root. Two of the internal phloem patches arise just below the cotyledonary node, the other two just below the node bearing the first pair of epicotylary leaves. She states that the internal phloem is an acquired characteristic of the plant, and that it has probably been developed to supplement the external phloem in the discharge of its functions.

WORSDELL (**25**), after an investigation of the anatomy of *Cucurbita*, *Acanthosicyos*, etc., concludes that "medullary phloem represents . . . . a vestigial structure, the remnant of a former system of medullary vascular bundles in which the xylem has disappeared." This phenomenon has been retained solely because of its rôle as a useful adjunct to the vascular system. The author refers to its development at a later period than the external phloem as an argument in favor of its vestigial character. He regards the bicollateral bundle of the Cucurbitaceae as a compound structure consisting of two distinct vascular bundles, of which the inner has lost its xylem. He concludes that the cucurbitaceous stem represents "the vestige of a former ancestral scattered system of bundles, such as obtains in monocotyledons, of which only two series or rings remain in perfect condition, the rest appearing in the form of rudimentary external phloem strands, internal phloem strands, and medullary bundles or phloem strands."

Recently the writer learned of the appearance of a work entitled "Die Cucurbitaceen" by ZIMMERMANN (**26**). A copy of the work has just been received, but it is impossible at this time to make any detailed reference to any part of it. From a hurried examination, however, it is concluded that the plants therein described are entirely different from those here described, and that the investigations follow different lines. The only possible exception to this statement seems to be that ZIMMERMANN has noted the occurrence of extrafascicular bundles in *Momordica*.

Histology of axis in seedling types

The writer has studied in special detail the early histology of seedlings in *Luffa aegyptiaca*, *Cucumis sativus*, *Cucurbita Pepo*, and *Momordica balsamina*. Serial sections of each of these were made from the upper part of the primary root, through the "heel" region where the root merges into the hypocotyl, and thence upward

to varying heights along the hypocotyl. The order of structural advance and complexity seems to be in the order above indicated, and hence *Luffa* will be described first.

It is worthy of note that all of these seedlings show a pronounced tetrarch bundle system in the root (fig. 1), the usual condition observed in dicotyledons. Certain strong similarities seem to justify the grouping together of *Luffa* and *Cucumis*, and *Cucurbita* and *Momordica* for descriptive purposes.

Luffa aegyptiaca and Cucumis sativus

The radicle of seedlings about ten days old showed a tetrarch bundle system, with two of the xylem masses slightly larger than the alternating two. This is noteworthy in relation to future procedure. In the two larger areas, three or four large, strongly marked, pitted vessels stand out conspicuously. These are surrounded by maturing wood fibers, which in turn are continuous with somewhat attenuate patches of spiral tracheae that tip the arms. The slightly smaller alternating xylem radii show a few small, somewhat irregularly disposed vessels among a few xylem fibers, while the arm is terminated by small patches of spiral tracheae as in the larger two.

About 1 mm. higher, in the center of the root, a core of delicate cells appears as a developing pith accompanied by a slight moving apart of the two pitted vessels and of their surrounding elements in each of the two larger xylem patches (fig. 2). Slightly higher, the four to six conspicuous pitted vessels with their surrounding xylem elements, seen in the two larger xylem masses, move still farther apart, while the pith tissue is considerably enlarged. As this goes on, the two smaller alternating patches become reduced to little more than two patches of spiral tracheae (fig. 3). Still higher, increase in the amount of pith tissue, continued moving apart almost to the separation of the conspicuous pitted vessels and the elements surrounding them, and a commencing division into halves of each of the smaller masses are all observed to be proceeding simultaneously (fig. 4). The four divergent pitted vessels with the xylem tissue surrounding them is shown below to represent the four divergent primary bundles in the lower part of the hypocotyl, while the spiral tracheae of the two arms and the divided spiral

tracheae of the smaller bundle masses will in time unite in front of the pitted vessels, as the normal protoxylem constituents of the stem bundles. This is shown by sections at the extreme base of the hypocotyl. Here, as shown in figs. 5 and 6, the two large xylem masses have now separated along with their surrounding elements as four distinct masses, although the spiral tracheae at the tips of the arms of these are still intact. The now split spiral tracheae, which made up the smaller alternating bundles, move toward and eventually fuse side by side with or internal to the pitted vessels and related tissue. Thus there are left two lines of radiating cells connecting the pith with the cortex. From this stage onward they become the two earlier formed of the ultimately four broad medullary rays of the lower hypocotyl. Slightly higher in this hypocotyl, the two patches of spiral tracheae in the now initiated meristeles become separated, half of the spirals in each arm moving alongside and then gradually in front of the pitted vessels, where they ultimately combine with the corresponding patch from the now broken smaller masses to constitute the internally placed protoxylem of each of the four bundles.

By this time the pith tissue has increased in amount, and the four meristeles have been pushed apart. Up to this point, the four now fully constituted meristeles show no indication of internal phloem, but have a well marked line of cambium, as well as adjacent cambioid cells, lying between the outer part of the xylem and the inner part of the phloem. From this time onward the cambium adds to the depth of xylem and phloem. About the level of the outprojecting heel, a line of nucleated cambial cells becomes continued inward around the sides and to the front of each bundle, so as to envelop each bundle in what might strictly be termed a "perixylary" cambial ring. The cells of this cambium along the sides of the bundles remain inactive for some time, but, as is indicated later, they become of great importance and functional activity in the aging stems. On the other hand, the cambium established in front of each of the four bundles starts active division, with the resulting formation of rudiments of internal phloem. Sections cut in a region slightly higher than the heel, therefore, show a well marked internal phloem to each bundle.

In the formation of this perixylary cambium, even at this early stage, phloem tissue becomes extensively developed along the lateral faces of the xylem, so that at times the bundles approach the concentric type (figs. 7–9).

At a distance of about 5 mm. above the level of the heel, a cross-section of the hypocotyl shows four widely separated bundles, each with a well developed cambium and external phloem, both of

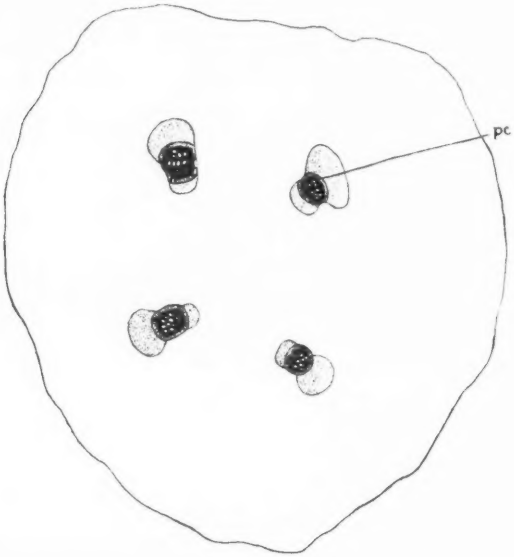

FIG. 1.—Diagrammatic transverse section of lower seedling hypocotyl of *Luffa aegyptiaca*, 5 mm. above level of "heel," showing four bicollateral meristeles each with well developed perixylary cambium (*pc*) and internal phloem; ×300.

which are more or less continued inward along the sides of the xylem, and also a pronounced area of internal phloem that is being increased by the activity of the inner portion of the perixylary cambium (text fig. 1).

As cross-sections are made at higher and higher levels of the greatly elongated hypocotyl in a seedling that is six to eight days older, one of these four bundles is observed gradually to split radially, at times equally, sometimes into a decidedly smaller and

a larger half. Still higher, and usually toward the opposite side from the bundle which had just split, another bundle undergoes a similar fission. Six bundles of rather unequal size, therefore, may be observed about the middle of the hypocotyl. Still higher, one bundle after another undergoes radial fission, until ten have been established about 3–8 mm. below the insertion of the cotyledons (text fig 2). As yet it is impossible to distinguish five larger and more internally projecting bundles from five smaller ones more externally placed.

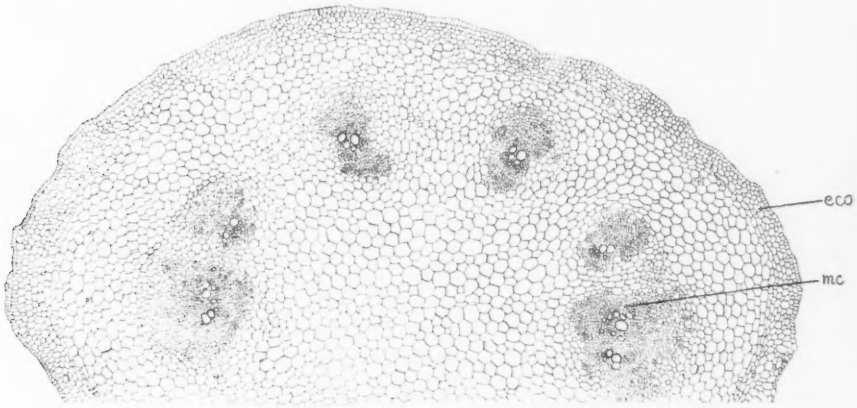

FIG. 2.—Transverse section of upper middle hypocotyl of seedling *Luffa aegyptiaca*, showing seven of ten vascular bundles: *eco*, exocortex; *mc*, medullary cambium (note radial splitting of bundles); ×60.

After giving off branches into the cotyledons, the ten bundles are continued into the lower part of the developing epicotyl, but, as this lengthens and matures, five alternating bundles become decidedly enlarged and project markedly into the pith, while the remaining five show less vigorous growth and soon are of smaller size as well as more external in position.

In the development of the seedling of *Cucumis sativus*, essentially the same mode of procedure is observed as in the preceding. The monostelic mass of each radicle, as it approaches the hypocotyl, splits into two larger meristelic masses, that include all of the large pitted vessels, and two alternating, decidedly smaller ones in which

the pitted vessels are relatively smaller. Separation of the larger ones into halves and later of the smaller ones follows, and the large permanent pitted vessels are contributed by the larger meristeles, while the smaller meristeles, in their halves, contribute along one side of each meristele the small pitted vessels and spiral tracheae which, from a lateral position, gradually move toward the front of the meristeles, as do the spiral tracheae of the opposite side that were contributed by the larger meristele masses.

Cucurbita Pepo and Momordica balsamina

In contrast with the last two, the types now to be described behave in what seems to be a more balanced manner. Sections of the radicle a few millimeters below the heel show an evenly balanced tetrarch monostele. Especially to be noted in *Momordica* is a strongly developed area of hard bast external to each phloem mass (figs. 10, 11). In passing toward the hypocotyl, the four radial and similar monostelic masses gradually separate with the increasing development of pith. Simultaneously the spiral tracheae divide into pairs, which later separate, and leave between them lines of cells which connect with the pith, and henceforth constitute the primary medullary rays.

Each half-mass of spiral tracheae gradually places itself alongside of and ultimately grows in front of the pitted vessels. Slightly higher, the two patches of spiral tracheae derived from adjacent arms, converging and moving in front of the pitted vessels, unite to form a single protoxylem mass. In this case the four primary parts of the root monostele, as they rise upward to become separate bundles in the hypocotyl, retain the pitted vessels intact in position as four conspicuous masses. Splitting of the four arms gives rise to four pairs of adjacent patches of spiral tracheae. The halves of each pair become more and more separated through prolongation, from pith to phloem, of cell rows that become the primary medullary rays.

Up to this point, and even 1–2 mm. higher, each of these four bundles is solely of the collateral type, and is entirely devoid of any trace of internal phloem. About the level of the "heel," two simultaneous changes are noticed to take place in *Cucurbita Pepo*.

The bundles start in somewhat uneven manner to subdivide, so as to give rise to six, seven, and eventually eight meristeles that become fully constituted as such at, or slightly below the level of, the upper surface of the "heel." At the same time a perixylary continuation of cells from the normal bundle cambium inward to the front of each bundle develops, and quickly initiates the first rudiments of an internal phloem. All of these tissues participate in the division of the four bundles into eight. About 5 mm. above the "heel," one of the eight bundles further undergoes splitting, so that nine bundles, arranged in a ring but of somewhat varying and unequal size, may be encountered. Slightly higher, splitting of another bundle gives rise to the mature ten, seen through three-fourths or more of the length of the hypocotyl and persisting upward beyond the cotyledons.

In *Momordica* a conspicuous patch of hard bast, that almost or quite equals the soft bast in extent, is present throughout the entire length of the hypocotyl, and is continued upward into the developing epicotyl (fig. 12).

Histology of mature plants

1. Trichosanthes colubrina

Near its apex the young stem shows an epidermal layer with long glandular capitate hairs of varying length. These persist over the stem through several internodes, but are gradually shed. Internal to the epidermis the cortex shows a collenchymatous layer, rather narrow between the five strong ridges that traverse the stem lengthwise. This collenchyma swells out into five strong prominent strands that evidently form the mechanical support for the young shoots. Subjacent to this is a narrow chlorenchymatous zone, one to three cells deep, continuous around the stem and forming a mediocortex. Internal to this is an equally continuous zone of tissue that very early becomes lignified, and is two or three cells deep, the whole acting as a fibrous strengthening zone. This has generally been termed the mediocortex, but its continuity, its subsequent behavior, and the relation and aspect of the cells within that surround the bundle, all indicate that this should be regarded as a sclerenchymatous endocortex. Within it is a nearly uniform

mass of ground tissue in which are imbedded typically ten vascular bundles as island masses. Hitherto the outer part of this, which consists of about three layers, has been viewed as an endocortex. Subsequent behavior and relation to the fibrous zone without lead the writer to regard this as conjoint endodermis and pericambium, the latter in this case being of several layers, as not infrequently happens in dicotyledons.

The fundamental tissue in fresh preparations has a strikingly similar appearance throughout in its cells, whether being in position or relation an endodermis and pericambium outside, or as medullary rays between the bundles, or as pith tissue internal to these.

The vascular system consists typically of ten bundles, five smaller external ones in line with the stem ridges, and five more internal, and (in part at least) larger bundles alternating with the smaller (text fig. 3). Two of the five inner bundles are often smaller, at times greatly smaller, and of more simple structure than the other three. This peculiarity, with attendant later developments, persists into the oldest part of the stem. Each bundle shows a rather small external protophloem, a cambial and cambioid zone within, a protoxylem of one to three rows of spiral tracheae and companion cells, and most internally an internal phloem that equals or even exceeds the external in width.

In an older stage, about five internodes removed from the stem apex, several interesting and suggestive changes have arisen. By growth expansion of the stem, the epidermis and collenchymatous cortex become stretched and compressed, many of the glandular hairs tend to wither and fall, and the chlorenchyma cells of the mediocortex increase somewhat in size and deepen in color. The scleroid endocortex shows a marked change and relation, however, for it becomes ruptured, mainly in line with the outer ends of the medullary rays, largely due to proliferation of cells of the pericambium at these points. This proliferated tissue forms somewhat soft, quadrangular cells that more or less interrupt the continuity of the rounded pericambial cells. Rupture of this sclerenchymatous endocortex has doubtless also been hastened by a great increase in size of the inclosed tissue, and especially of the vascular bundles. Each bundle has developed from its normal cambium a considerable

internal patch of metaxylem composed of wood fibers, large pitted vessels, and a few simple, slightly thick walled wood cells. This cambium, on its outer face, has developed a conspicuous amount of metaphloem with prominent sieve tubes that are in connection with phloem cells. The internal phloem has also developed markedly through activity of the internal part of the perixylary

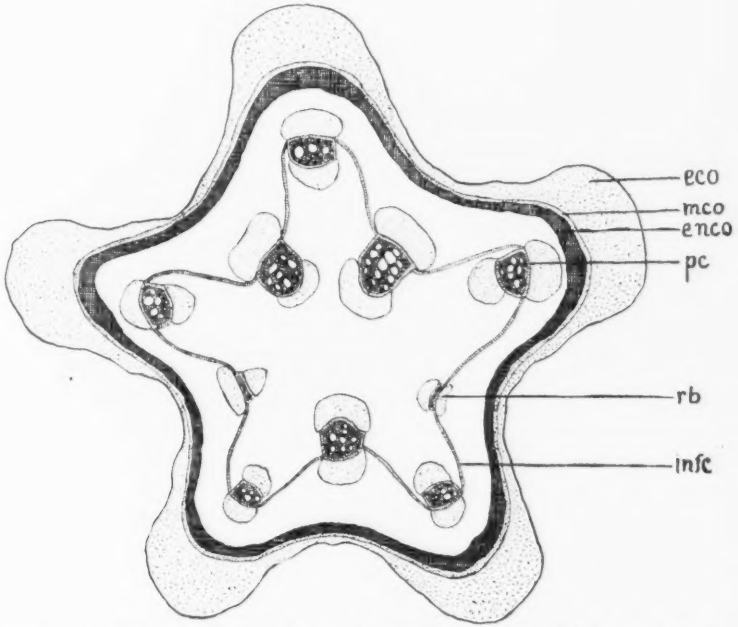

FIG. 3.—Transverse section of stem of young *Trichosanthes colubrina* near apex, showing two rudimentary inner bundles (*rb*), sinuous cambium, perixylary cambium (*pc*), and differentiated ectocortex, mediocortex, and endocortex regions (*eco*, *mco*, *enco*); ×45.

cambium, or, as the writer would term it, the "prefascicular" cambium. This internal phloem in amount may be half to as much in depth as the external phloem. By this time also the protoplasmic and proteinaceous constituents of the sieve tubes have become abundant and rich in substance, so that these stain deeply with the methyl blue stain, especially where these substances are

heaped above a sieve plate. With this method of staining the continuation inward along the sides of the bundles from the outer cambium to the prefascicular cambium can clearly be traced. The lateral cambial lines are dormant in their activity as yet.

In a still older section that is 5 mm. wide, about two weeks matured from the growing apex, further advances are observed. The endocortical sclerenchyma has become still further split into more widely separated parts, owing to growth and possible proliferation of pericambial cells. Marked widening in all of the bundle zones is observed. Each as yet immature pitted vessel shows at present and retains for a long period after this a prominent nucleus and nucleolus. By this time marked inequality in two of the five larger inner bundles may be very striking, for, while the other three now show large internal phloem areas with abundant sieve tubes, a fourth has a very small patch of internal phloem, and a fifth scarcely shows a trace, or at most a very small area of six to eight cells (fig. 13). The larger three, also, have pressed strongly into the pith region. Along the sides of all the larger bundles, the lateral parts of the perixylary cambium are laying down somewhat irregular, more or less isolated, phloem tissue, so by this time it may be said that cambial activity and phloem production have already started in a somewhat discontinuous but concentric manner around each bundle.

When a slightly older stage has been reached, the three inner bundles, occasionally even the outer bundles, show progressive development of secondary medullary rays from the normal cambium. Along one or both sides of such rays a cambial line of cells has been left which may already have developed faint beginnings of lateral phloem patches (fig. 13*mph*).

Further, the lateral phloem areas along the sides of two adjacent bundles may now be observed to have developed oblique or almost transverse interfascicular phloem bridges, linking up the lateral phloems of distinct bundle masses. Equally marked connections are here and there established between the external phloems of adjacent bundles.

By this time the upper part of the hypocotyl shows a closely similar development to the condition last described. Most note-

worthy in this region are the numerous connecting strands of phloem that constantly pass from the phloem of adjacent bundles across the medullary rays, and equally that pass from the internal phloem along the medullary rays outwardly or crosswise to join with the internal phloem of another bundle. Equally striking are the extensive transverse or oblique bridge connections established between the metaxylems of different bundles (fig. 14). Morphological and physiological connection is thus suggested for the metaxylem as well as for the lateral phloem areas of adjacent bundles.

Even more remarkable is the extensive cross connection established between the external phloems of adjacent bundles. Still another feature has now become especially prominent, although traceable in younger or higher stem sections. During development and differentiation of the cells derived from the pericambium, certain of these cells in line with each other and with the exterior ends of the medullary rays make direct connection with the most external sieve tubes of the phloem of each bundle, and also connect with and affect the cells of the soft mediocortex, so that these become modified into that system of sieve tubes which FISCHER (3) has designated "extra-cyclic sieve tubes." By this time the stem has matured through three weeks to a month of growth. About two feet removed from the apex, such growth has proceeded in the phloem of the axis that not only is there an extensive internal and external phloem, but lateral phloem masses have formed along the sides of the metaxylem which may almost or quite connect the external and internal phloems. These lateral areas by oblique or transverse diverticula, crossing the medullary rays, may directly connect with one another. Like connection between the phloem of adjacent bundles is even more extensive, while external ramifications from the external phloem into the mediocortex are fully established.

It will be seen from fig. 13 that the condition of bundle inequality already noted in the young stem has persisted and become greatly accentuated in the nearly mature plant, that is, in the older part of its stem.

POTTER (11) calls attention to the formation of secondary medullary rays in the plants he studied and regards their presence

as a somewhat noteworthy feature in the history of cucurbits. In *Trichosanthes* and other short-lived herbaceous types, however, secondary medullary rays are formed by the bundle cambium in one to three of the stronger bundles at least. Not only so, but in the largest bundles of a four to five months old stem, tertiary medullary rays of fair depth may have developed. The later formed medullary rays pass out to phloem tissue of similar age. In this manner, all parts of the increasingly deepening xylem of the bundles are kept in close contact physiologically with the elaborated sap passed down through the external phloem.

As transition is made to the oldest and thickest part of the stem in a mature plant, the structure is fundamentally the same as that already detailed. As one approaches the region of the decayed cotyledons, the interfascicular xylem and phloem connections have become still more extensive and intimate. The secondary changes exhibited in the hypocotyl, as compared with its structure in the seedling state, are the most conspicuous features in the history of the plant, and are of such a nature as to cause one, somewhat metaphorically, to liken it to the central exchange in an extensive telephone system.

A hypocotyl that is from two to three months old shows considerable reduction in size of its sclerenchymatous endocortex. This appears now only as small and scattered patches of lignified tissue. The bundle relations are of great and special interest, for, as compared even with the lower part of the epicotyl, this region shows a widespread ramification of sieve cells that extend from the external phloem outward into the mediocortex; at times, even to its outer margin. Through pericambial activity, mainly in line with the outer ends of the deepening medullary rays, abundant sieve cell connection is established between the external phloems, so that not infrequently these may be linked together into one common phloem system. In addition, during the steady growth of the hypocotyl, abundant bridges of sieve tubes pass inward from the normal phloem; in part to interconnect from bundle to bundle, and in part to establish connection with masses of lateral phloem already developed along the radial faces of the bundles. These lateral patches form frequent interconnections across the medullary

rays, and not infrequently show connections with the internal phloem. In passing, it may be observed that one or more of the bundles have developed such cambial activity along the one or both sides of the xylem, that a broad plate of typical phloem may arise that extends continuously from the internal to the external phloem.

As passage is made toward the lower part of the mature hypocotyl, the elements of the wood exhibit fewer lignified elements, so that in some bundles patches of wood fibers surrounding one to four pitted vessels may be imbedded in the midst of an extensive area of thin walled parenchyma. Furthermore, the entire sieve tube system now reaches a climax of complexity, abundance of sieve elements and branchings. Correlated with this are the frequent cross connections between the xylems of adjacent bundles, and in these the pitted vessels, exposed in their pitted faces, form striking objects.

As transition is made to the summit of the mature root, the interlacing connections become still more complex, so that a confused tissue pattern only is discernible. Especially is this so if one or more side rootlets start from the main root. While this is proceeding the lateral plates and internal phloem masses of the perixylary system gradually break up and retreat toward the exterior, the latter having previously come together as a central mass of tissue. Deeper down this thins out, leaving in the lower part of the root six to four bundles that meet in the center by their protoxylems, but which externally have become more and more deeply separated by the increasingly wide medullary rays.

2. Luffa aegyptiaca

Study of soft young stems of *Luffa* about a foot in height, about the second epicotylary internode, shows a sharply pentagonal outline, with epidermis, collenchymatous, chlorenchymatous, and as yet immature sclerenchymatous cortical zones continuous around the stem. Imbedded in the fundamental tissue are typically ten bundles, five of which are internal and five markedly external. As in *Trichosanthes*, however, it frequently happens that at least one of the internal bundles is decidedly smaller than the other four, a

peculiarity which may persist into the mature stem. Already, far down in the hypocotyl, a prolongation inward of the perixylary cambium has on its inner face given rise to an internal phloem area for each seedling bundle, but in this type a semi-crescentic internal phloem has already originated that may be half as large again as the flat external phloem. Frequently, also, the lateral rows of cambial cells that connect the external and internal cambiums are readily discernible. Thus again is constituted a perixylary sheath. The young bundle apart from this deserves no special description.

If now, as for *Trichosanthes*, successive sections be made of a mature plant about four months old, starting with the third or fourth internode from the apex and gradually proceeding backward, the following features might be emphasized. The stem outline is not merely pentagonal as in the seedling, but, owing to luxuriant development of the collenchymatous cortex along five lines, it exhibits five ridges that alternate with five grooves. The lignified endocortex has already developed thickened walls in its five to seven layers. The one small inner bundle usually shows no internal phloem at this stage. A second bundle of the inner five may also be reduced in the amount of xylem and phloem, and not infrequently has no internal phloem. Sections made about the eighth internode from the growing apex still show a continuous zone of lignified endocortex, but in relation to the future behavior of this tissue and the outward passage of sieve tubes into the mediocortex, it should be noted that narrow radial patches of richly protoplasmic cells grow into the six inner lignified cell layers of the endocortex, thereby leaving for a time only one or two external lines in continuity.

A feature shown at this time in *Luffa* is the formation of a continuous cambium ring of highly sinuous course. As shown in fig. 15, one can readily trace between any two bundles three to four flattened, highly protoplasmic nucleated cell rows, which are continuous with similar cells within the bundles. The central row is a cambium, while those on either side are young cambioid cells. This histological detail bears out and further extends POTTER'S findings in *Cephalandra* (11), that the five outer and five inner bundles are the slightly displaced members of one ring, typical for dicotyledons. This also negatives WORSDELL'S (25)

hypothesis that primitive cucurbits started with numerous irregularly disposed bundles.

Sections made through the twelfth to fifteenth internode from the apex show a greatly stretched epidermis, exocortex, and mediocortex as a narrow zone. The endocortex has become broken up into lignified patches immediately exterior to each bundle. Marked growth has taken place in all of the vascular bundles. The striking difference of the interfascicular cambial cells as compared with those of the intrafascicular stretches deserves special notice. While the latter are small, flattened, and truly cambioid, the former are large, finely protoplasmic, and nucleated cells of quadrangular outline but with extremely thin walls. By subdivision of these, cells arise externally and internally which add to the depth of the medullary rays, and also to the lateral lines of perixylary cambium. The lateral portions of the perixylary cambium, as in *Trichosanthes*, have now started considerable lateral additions to the phloem, especially along the sides of the larger and deeper bundles (fig. 16). Secondary medullary rays, about ten to twelve cells in width, have been laid down, and these divide more or less completely the original phloem of each bundle into halves. The internal phloem masses have so grown and pushed inward that the area formerly occupied by the pith is now filled. Occasionally, also, oblique sieve tubes and companion cells may stretch outward from one or more of these to connect with the corresponding elements of the medullary phloem.

Between the last condition of stem section and the mature epicotyl one or two internodes above the level of the cotyledons, important growth activities have taken place. Each bundle, with the possible exception of the original inner one of the five, is now a broad wedge-shaped mass, bounded on either side by the primary medullary rays and the lateral zones of perixylary cambium. Deep secondary medullary rays, tertiary, and occasionally short quaternary medullary rays extend into the phloem. All of these are about eight cells in width. The outstanding features of growth, however, are now centered in products of the lateral and prefascicular cambial tissue. As compared with the rather limited amount of medullary phloem described in the previous paragraph, the lateral cambium

has actively proliferated additional phloem tissue, generally in localized masses, and also has cut off cells that simulate, and evidently function as medullary plates at right angles to each primary medullary ray, termed by the writer "admedullary rays." Not only so, but secondary lateral cambiums, peculiar to each patch of these, have laid down internally masses of tertiary xylem of varying size, composed in great part of thick walled fibers, but including also one to four pitted vessels. These tertiary bundles produced by the perixylary cambium the writer has designated "transmedullary bundles." Even more remarkable are the changes effected in connection with the internal phloem masses. Each of the nine or ten prefascicular cambiums, that formed for a time only internal phloem, has laid down between itself and the protoxylem a deep mass of tertiary xylem. Thus are formed the obcollateral bundles (text fig. 4). Furthermore, the xylems of the transmedullary bundles and the xylems of the internal obcollateral bundle masses throw out frequent oblique or transverse branch connections that fuse with one another.

Toward the base of the secondary medullary rays, a limited amount of cambial activity gives rise to small areas of sieve tubes and companion cells, termed "intraxylary phloem" by the writer. The result of this enormous internal growth activity is that the original pith tissue or pith cavity becomes completely filled up with large internal obcollateral pith bundles, which, however, still retain connection with the protoxylems of the bundles.

Although up to this point no attention has been called to the external ramifications of the sieve tubes and companion cells in *Luffa*, it should be said that even in sections of the young stem like the second or third described, ramifications are sent out from the external phloem which at times cross to make connections with adjoining bundles on either side, but also, passing outward, begin to unite with cells of the mediocortex or to insinuate themselves between the masses of broken up and now discontinuous endocortex. Resemblance, therefore, to the conditions already described in *Trichosanthes* is perfect.

In passage downward through the hypocotyl (which may be nearly an inch in diameter) conditions become even more exagger-

ated and complicated. More and more copious anastomoses of the external phloem patches may be effected with each other, and may abundantly be sent out into the mediocortex. Longitudinal, oblique, and radial diverticula may also connect the xylems of the

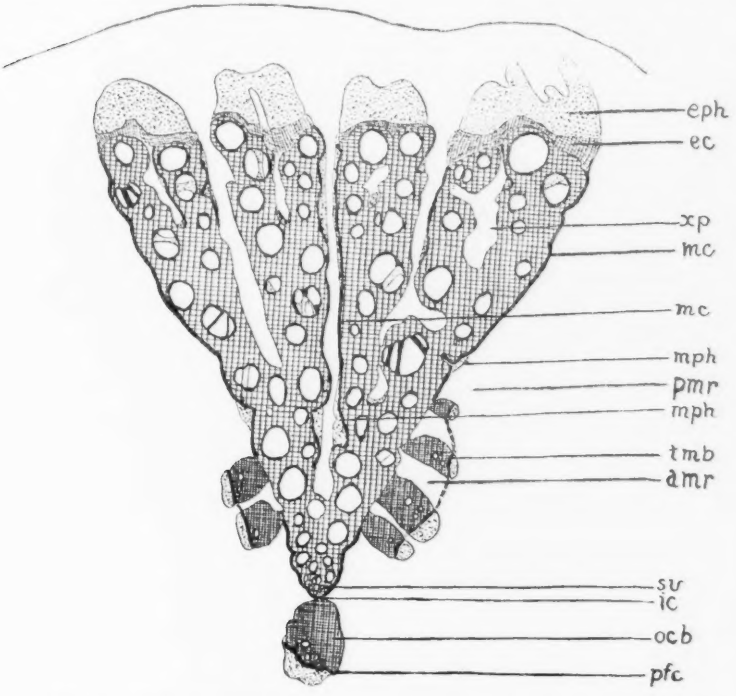

FIG. 4.—Transverse section of hypocotyl of mature *Luffa aegyptiaca*, showing vascular bundle whose internal phloem has become obcollateral bundle (*ocb*) by addition of secondary xylem produced by prefascicular cambium (*ic*); inner areas of medullary phloem by addition of secondary xylem produced by sections of medullary cambium have become transmedullary bundles (*tmb*), separated by admedullary rays (*amr*); medullary phloem formed along secondary and tertiary medullary rays (*mph*) by medullary cambium (*mc*); ×16.

ten bundles. The transmedullary bundles here reach their greatest size, as do the admedullary rays between them. The obcollateral bundles that primarily originated as internal phloem masses now frequently become wholly separated from the protoxylem.

Finally, as transition is made to the root, thinning out and disappearance of these bundles takes place, and equally of the transmedullary bundles, until, in the root proper, four convergent wood wedges unite with each other by their internal faces or apices.

Thus it is evident that the hypocotyl is the most complex region of the axis, and in the extensive and intricate ramifications and unions of the separate bundles (equally in their phloem and xylem constituents) there is a remarkable provision made for the passage of abundant crude sap upward along the xylem, and of elaborated sap downward in the greatly developed phloem.

3. Citrullus vulgaris

This excels all other members of the family in length of axis, in luxuriant growth of side branches, and in size of hypocotyl and immediately connected region above and below. Sections of the main axis or of a side branch, about three internodes removed from the stem apex, in a plant about four months old, show fundamentally similar detail to that of *Luffa* in its epidermis and cortex, except that the continuous lignified endocortex is here only two or three layers deep. Internal to the endocortex is a thin walled epidermis of rounded cells, and internal to this in turn is the pericambium.

The same inequality in bundle size that has previously been described for *Luffa* is observed in *Citrullus*, and each bundle develops the typical perixylary cambium. Ten radiating groups of thin walled cells at the back of and in line with the ten medullary rays are also richly protoplasmic, and mark the future areas where abundant cambial growth in and around them will cause splitting of the lignified endocortex. The pith tissue in *Citrullus* early breaks down in large measure.

While account has not been taken in any of the preceding descriptions of the presence of tyloses, it might now be said that this is an extremely common and striking feature in most of the cucurbits studied. The large pitted vessels in the earlier stages of their maturation frequently become invaded by surrounding thin walled cells. Each tylosis cell is provided with active protoplasm, and a large evident nucleus, and a shining nucleolus. These cells evidently

remain for a considerable period as a living, spongy pseudoparenchyma that more or less fills up the vascular cavities. Of all the types studied in this family, *Citrullus* shows this feature most strikingly. Already, in the young sections just described, the inner two or three of the large pitted vessels among the eight or more now formed show extensive tylosis. Gradually, as the bundles increase in depth and additional pitted vessels arise, similar tylosis is seen proceeding in them. It seems to the writer, however, as if certain of the vessels in the younger stems were exclusively or mainly invaded in this manner, but with increasing age a progressive development of tylosis goes on, so that in stems about four months old and about an inch in diameter, nearly all of the now abundant pitted vessels are blocked up with tylosis tissue. Moreover, if one may judge from the staining reaction of the protoplasmic contents and the persistence of the nuclei, this tissue seems to remain alive and may importantly function in the distribution of crude, and may be even of elaborated sap.

In sections taken through the eighth to tenth internode, the radiating groups of cells, described as projecting into the lignified endocortex, have so enlarged and multiplied that they have caused the endocortex to break up into ten distinct sclerenchyma plates. A large celled interfascicular cambium is now fully established, and from this time on steadily cuts off large medullary ray cells on its inner and outer faces. Of the large inner bundles, a fourth in addition to the preceding three may be nearly or quite equally developed, while an extreme rudiment of the fifth (that appears only as a small patch of phloem tissue) may still remain.

The lateral portions of the perixylary cambium may already have formed a slight amount of lateral medullary phloem. As a rule this consists only of undifferentiated cells or patches of smaller, somewhat angular phloem cells. Occasionally one can observe one or more sieve tubes, which represent the beginnings of what later become the transmedullary bundles.

Selecting next an internode that is a centimeter in diameter, one observes immediately external to the bundle phloem two layers, occasionally widening to three, of rounded cells that represent endodermis and pericambium. Between the lateral exteriors of

the bundles and the extremities of the rapidly deepening medullary rays, proliferation of the pericambial tissue has given rise to as many as eight layers of more or less cubical cells. The vascular bundles have increased markedly in size, and, if the stem or lateral branch has grown rather freely on all sides, the five inner bundles may all be represented as well as the five outer, for, as discussed more in detail under the physiological section of this thesis, the relative size to which the inner five bundles of the axis may attain seems to be largely conditioned by the dorsiventral relations or by mechanical strains along the plant axis. As a rule, four at most of the five inner bundles are almost or quite equal in size, a fifth being often decidedly smaller and more external. The inner bundles also are on the average three-fourths to as much again larger than the outer bundles. The external phloem is now greatly increased in amount over the internal, being three to five times greater.

The sieve tubes in *Citrullus* are probably the largest and finest studied by the writer. Those of the external phloem are on an average as large again as those of the internal phloem. The external and internal segments of the perixylary cambium are more strikingly regular and cambium-like than in any other type studied. From the latter portion, a steady amount of phloem is laid down by differentiation of the cambioid cells. Furthermore, the commencing formation of lateral transmedullary bundle rudiments as described in the previous paragraph has considerably advanced. It is noteworthy, however, that at the present stage, and even in somewhat larger and older axes, transmedullary bundle development only proceeds along one side of two or three of the inner bundles (text fig. 5).

The earliest formed and internal part of the xylem of *Citrullus* consists almost wholly of indurated wood fibers and large pitted vessels that soon become invaded by tylosis cells. As compared with other types already studied by the writer, however, from the age of about a month onward the xylem becomes soft and largely cellular, with only scattered irregular masses of indurated elements and pitted vessels. This cellular condition is due in part to the rather irregular and broad spreading character of the secondary and succeeding medullary rays, but in largest measure it is due to

extensive areas of xylem tissue that remain in the parenchymatous state instead of becoming fibers or large vessels.

Up to this stage little, if any, indication has been shown of connections between the external phloems or other tissue of the bundle. Some of the cambioid derivative at the ends of the medul-

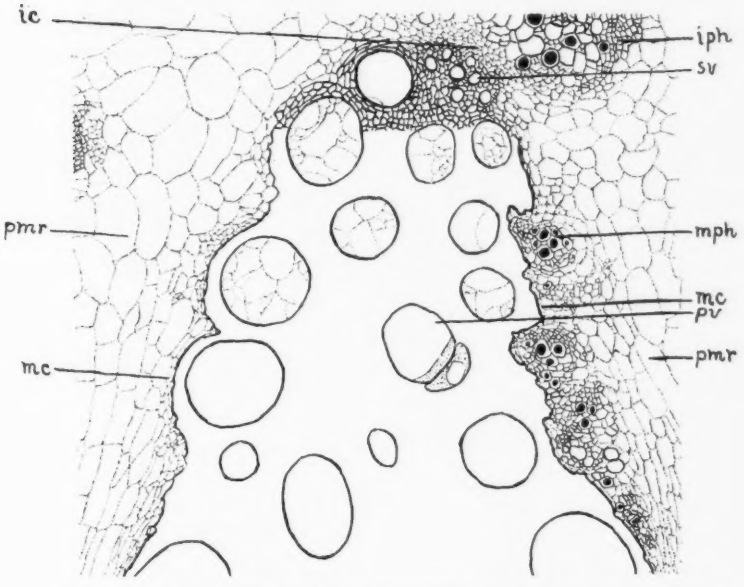

FIG. 5.—Transverse section of branch of mature *Citrullus vulgaris* near hypocotyl; inner part of one bundle showing large areas of medullary phloem (*mph*) formed by medullary cambium (*mc*) along lateral faces of metaxylem of bundle; note almost entire absence of medullary phloem on opposite face of xylem: *ic*, prefascicular cambium; *iph*, internal phloem; *sv*, spiral vessels; *pmr*, primary medullary ray; *pv*, pitted vessel (details of xylem tissue omitted except at extreme inner part); ×50.

lary rays shows conversion into sieve cells with well developed sieve plates, that often in one place appear on edge, in another oblique, or again in full face expression with the sieve pores perfectly exposed. From this stage onward a steadily progressive advance in such oblique or transverse anastomoses may be traced; but further, the beginnings of differentiation in cells that connect

with the mediocortex constitute the first beginnings of FISCHER'S ectocyclic phloem (3).

Passing next to stems or branches that are about 1.5 cm. in diameter, the deep development of secondary, tertiary, and even at times of beginning quaternary medullary rays tends to cause separation of the external and hitherto continuous phloem areas into two, four, or even six separate patches. Owing, however, to what the writer at present only can regard as a growing capacity in sieve tubes of the bundles and in medullary ray cells between the phloem masses, more and more extensive connections pass from phloem mass to phloem mass, and also outwardly into the cortex, so that FISCHER'S ectocyclic phloem is now in active process of differentiation. In the meantime, the internal phloem areas have been rapidly increasing in size through activity of the prefascicular cambium, but greatly more striking is the rapid increase now proceeding in the medullary phloem of nearly all the bundles. Along the sides of the secondary and tertiary medullary rays, or in cellular pockets left undifferentiated in the metaxylem, isolated phloem groups have appeared in quite irregular and scattered manner (fig. 17).

The highly parenchymatous nature of the xylem, as well as the irregular outlines of the secondary and later medullary rays, has now become a pronounced feature of the stem. It might be added that a steady formation from within outward of vascular tylosis tissue has so proceeded that a large number of the pitted vessels are now extensively filled. The extensive development that has gone on in the internal phloem areas, particularly in those of the inner bundles, has now caused extensive filling up of the area once occupied by the pith cells.

In still older stems, about the third to the fifth internode above the cotyledonary junction point, the stem diameter may be 2–2.5 cm. Extensive transverse bridges between all of the external phloem masses have now been effected through continuous growth of the phloem cells and sieve tubes from one bundle toward another. Into the numerous zones of cells that lie internal to the lignified endocortex, abundant ramification and prolongation have been effected, so that it can truly be said that there is now a broad

continuous zone of external phloem, made up of vertical, oblique, and transversely connected sieve tubes interspersed with companion phloem cells.

The only exception to this statement is where the large cells across the outer part of the primary medullary rays have so persisted in their function of distributing the elaborated sap transversely that few, if any, of the connecting phloem strands have succeeded in differentiating amid this transversely conducting cellular expanse. It might also be noted that the total bulk of phloem equals one-third to one-fifth that of xylem, account being taken of the zone of external phloem, the important lateral medullary phloem, and the eight to ten internal phloem masses. This is in marked contrast with the relative amount of phloem and xylem even in ordinary herbaceous plants, not to say in shrubs or trees, so that it seems to be another correlated and necessary factor in the enormously rapid growth and vegetative luxuriance of this plant. This question is discussed from the physiological standpoint later.

Such a condition is entirely in keeping with the extremely rapid growth and high nutritive capacity shown by *Citrullus*. About this level, also, bridges of xylem are occasionally laid down, connecting adjacent bundles, although as yet these are not nearly so numerous or wide as in the hypocotylary region next to be described. The accessory intraxylary phloem patches now have become more abundant and larger in extent.

The hypocotyl in a mature plant (one about four months old) measures about 3.5 cm. in diameter, and 15–20 cm. in length. The external phloem has now reached its maximum of growth, and through formation of successively intercalated secondary, tertiary, and succeeding subsidiary medullary rays, the original primary phloem mass has split up into 16–24 phloem wedges of varying size. These daughter phloem areas all communicate with one another by oblique or transverse sieve tubes, as well as by companion phloem cells. In the peripheral portion of the hypocotyl especially, the copious anastomoses of sieve tubes are most striking, and frequent passage of them between the peripheral sclerenchyma patches can be seen to take place around the entire axis. It is in the region occupied by the xylem tissue or the area

internal to it, however, that the climax changes have been effected that are now to be recorded.

The xylem in the hypocotyl region shows a more extensive cellular structure than does the oldest or any other part of the epicotyl. Frequent patches of two to six large pitted vessels surrounded by thickened wood fiber elements may be wholly surrounded by thin walled parenchyma. The formation of tylosis tissue within the vessels has here reached a climax. It is in the marvelous development of perixylary and intraxylary phloem, however, that the hypocotyl is preeminently distinguished. As to the former, one may notice that just beyond the huge patches of internal phloem there have developed frequently from twelve to twenty lateral medullary phloem masses, through the activity of the lateral sections of the perixylary cambium. In many cases, especially toward the interior, these phloem islands may be quite separated from one another by admedullary ray cells running at right angles to and meeting with the primary medullary rays. More externally, however, instead of isolated phloem areas, deep tracts of phloem may be intercalated between a primary medullary ray and a xylem face.

The feature that eminently distinguished this plant from all other types studied consisted in the luxuriant development of intraxylary phloem in a most irregular and scattered manner through the xylem parenchyma or along the lines of the tertiary and subsequent medullary rays (fig. 17). The total amount of such tissue in each of the xylem areas varies considerably, and seems determined in part by the position of the bundles in relation to the strongest radiating branches in the epicotyl above. One wood-wedge may have rather small and scattered patches toward one side of the area and extensive masses toward the other. Some wedges may be rather wanting in this tissue, or have only small patches of it; while an adjacent xylem mass may have large and abundant phloem groups. Furthermore, the sieve elements vary considerably in size; some small sieve cells with transverse plates show only about ten pores, others again may almost equal the sieve plates of the outer phloem, and then show fifteen to thirty pores. Collectively the amount of accessory phloem thus developed must form a

valuable auxiliary to the external and internal areas. Here it may be added that these phloem patches not infrequently form anastomoses with each other.

Turning now to the internal phloem, each of the four or five masses belonging to the inner bundles has increased in the hypocotyl to an enormous degree, and even those of the four or five outer bundles are of large size. The inner ones deserve special attention, for in addition to retaining in an active state the prefascicular cambium that normally functions in the epicotyl, and which here has given rise to huge internal phloem tracts, a crescentic line of cambial tissue has developed around the inner face of each internal phloem area, which lays down a crescentic mass of xylem either as a continuous mass or as two or more masses that are separated by short medullary rays.

In transition from the thick hypocotyl to the root, it usually happens that several rather strong secondary roots pass off from the primary so that the size of the latter is quickly reduced. In nearly every point of structure, however, the details revealed in the hypocotyl are continued into the root tissue. Not the least conspicuous in this connection may be mentioned the wide and luxuriant peripheral phloem zone, the highly cellular xylem, the abundant vascular tyloses, and the rich intraxylary phloem. The highly complex internal phloem patches previously described gradually thin out and are absorbed, and contemporaneous with this the xylem wedges come together to form a continuous central tissue.

In brief summary of the details shown by *Citrullus vulgaris*, it might be said that it greatly excels all other types studied in the large size and soft succulent character of the xylem masses; in the wide, irregular, and abundant medullary rays (secondary, tertiary, etc., included); in the extreme size of the normal phloem; in the abundant anastomoses that prolongations of these bundles form between one another and into the endocyclic cellular tissue as well as (although to a less degree) into the ectocyclic; in the extreme activity of the entire perixylary cambium; in the huge internal phloem masses with their ultimately complicated obcollateral bundles that result from the activity of the prefascicular cambium; in the marked activity of the lateral perixylary cambial stretches

with resulting formation of transmedullary bundles; but most of all in the often extensive patches of intraxylary phloem that must add greatly to the capacity for rapid transfer and lengthwise distribution of elaborated food along the lower part of the stem axis and all parts of the root system. When one surveys the collective development and capacity of these constituent elements, it is safe to say that *Citrullus* greatly surpasses any other type of rapidly growing herbaceous plant. It is throughout the length of the hypocotyl that these complexities reach a climax of development.

4. Lagenaria vulgaris

Sections of the lower middle hypocotyl of *Lagenaria vulgaris* reveal differences in structure that are not shown in any of the preceding types, and which persist from the young state up to and including mature growth. Such a hypocotyl, in a plant about 18 inches in length, shows an external epidermis abundantly provided with short pointed hairs. Underneath this is a rather discontinuous zone of colloid exocortex, but, in contrast with *Citrullus*, *Luffa*, and *Trichosanthes*, a sclerenchymatous endocortex is here wholly wanting. The endodermis and pericambium together consist of two to four soft, thin walled cell layers, the inner of which lies directly against the bundle system. The bundles here are eight in number, and are disposed in a single ring, although four that alternate with the other four are recognizably larger and project slightly more into the pith. Furthermore, two of these inner bundles are decidedly larger than the other two, indicating that each of these will split radially and so give rise to the full complement of ten, seen in the upper part of the hypocotyl as well as typically in the epicotyl.

Each young bundle of the hypocotyl in the age indicated exhibits a patch of hard bast, the lignified elements of which readily absorb safranin stain and assume a bright scarlet tint. The zone of perixylary cambium can readily be traced around each bundle, and may even develop at this early stage slight indications of lateral phloem. A large lysigenous cavity exists in the pith, and that is bounded by two to four continuous zones of pith cells.

Very early in the growth of the hypocotyl and young epicotyl, crescentic cambioid cells originate that join on with the intrafascicular cambial sections. By this union a highly sinuous although continuous stem cambium can readily be traced. Through rapid division of the constituents, the medullary rays from this stage onward are rapidly deepened.

In passing toward the upper part of the hypocotyl, the two larger inner bundles undergo longitudinal splitting, and from this point upward ten bundles are typical. It not infrequently happens that one bundle becomes feeble in growth, or is even eliminated, but in connection with these large bundles, and of extreme interest in relation to the structure of the epicotyl as contrasted with the hypocotyl or the root, there appear external to them two crescentic endocortical masses of sclerenchyma. These are the beginnings of a typical endocortical sclerenchyma ring formed by gradual spreading of the two patches outward on either side, as transition is made to the epicotyl. It will thus be seen that the hypocotyl and epicotyl contrast markedly in two histological details. The eight to ten hypocotylary bundles each possess a strong heavily lignified patch of hard bast fibers, which are lacking in the bundles of the epicotyl. The hypocotyl, also, is wholly devoid of lignified endocortical ring, except for a rudiment toward the summit of the hypocotyl; while the epicotyl, throughout its entire maturing history, shows a continuous sclerenchymatous endocortical ring, which gradually splits into patches with the increase in size of the stem.

Sections through about the third or fourth internode from the growing apex show a continuous sclerenchyma ring that is about four cells deep. This ring surrounds an endodermis and pericambium of two to four layers of rounded cells. The perixylary cambial tissue that surrounds each bundle stands out prominently. Each internal phloem patch may be about the size of the external, or from such to about three-fourths as large. The lysigenous pith cavity of the hypocotyl has been continued upward, and is here as pronounced as in early growth of the seedling.

About the twelfth to the fifteenth internode from the growing apex, the endocortex has split into patches of varying size. Deep

secondary and less deep tertiary medullary rays have been inserted, and passing outward separate each external phloem area into four minor patches. Along the inner ends or sides of these minor rays a limited amount of intraxylary phloem forms, but this never compares in amount with that already described for *Citrullus*. Through activity of the lateral sections of the perixylary cambium, deep plates of medullary phloem arise that may stretch through three-fourths the depth of the adjoining radial faces of the xylem, but, as in most of the types studied, very unequal amounts of this tissue may arise on opposite sides of any bundle xylem.

In *Lagenaria*, each internal phloem patch has attained quite a large size during this stage, although in comparison with the external, the internal is only about one-sixth to one-eighth the area. Through steady expansion of the circle of pith cells and of the central lysigenous cavity, these internal phloem masses stand apart from all other constituent tissues of the region.

On reaching the upper part of the mature hypocotyl, the same climax complexity of structure is seen that characterizes those types already described. The pericambial tissue has divided to the extent of forming at least four encircling layers of cells. The isolated hard bast areas have somewhat spread out into oval masses. Each bundle patch of external phloem is now so extensive as almost to equal in size the collective mass of xylem.

Probably in no types studied does the perixylary cambium show more clearly than in this plant, and especially in its hypocotyl. Connecting externally with the normal cambium, it winds along the sinuous edges of the bundle, and in the mature hypocotyl not infrequently forms on its outer face a continuous mass of phloem that unites the internal with the external phloem. Unlike *Citrullus*, intraxylary phloem is here very feebly developed. The internal phloem areas are oval to circular in outline, and attain to rather a limited growth. So far as observed, neither accessory cambium, internal xylem, nor sinking in of the phloem patches into the pith takes place. Tylosis in the pitted vessels is of rather rare occurrence in this type.

In passing from the hypocotyl to the root, the internal phloem masses gradually attenuate in size, while the bundle apices fall together, but the extensive lateral medullary phloem plates per-

sist. Still lower, the eight bundle masses fuse into six and eventually into four wedges, while the lateral medullary phloem diminishes in amount.

This accessory tissue tends to persist for a considerable distance downward in the primary root as shallow irregular sheets of phloem bordering the faces of the xylem tissue.

5. Momordica balsamina and M. charantia

Sections made through about the fourth internode of a mature stem show a structure essentially the same as that already described for the upper part of the hypocotyl and the lower part of the epicotyl of the seedling. The ten (or occasionally nine) bundles usually show five larger inner and five alternating smaller ones, that may be externally on a level with the larger bundles or may slightly project beyond them. In this genus the cortex consists at most of only four layers of cells, entirely lacking in lignification. Internal to the cortex is an endodermis surrounding a pericambial layer, both of which lie immediately against the strong patches of hard bast peculiar to the bundles. The pericambium deserves emphasis here because of its significance in the future increasing growth of the stem. In *Momordica* a line of development and of secondary increase takes place which is entirely different from anything that the writer has observed in the other genera of Cucurbitaceae. It should be noted that internal to the level of the hard bast, and on either side of the lateral angles of the soft bast, two groups of cells undergo considerable lignification. Since these arise near the exterior corners of the soft bast, they might be termed accessory hard bast. Occasionally, however, only one may develop or both may be absent. Generally they are very conspicuous and persistent structures.

Above this stage also, along the lines of the medullary rays and inward even to the pith, practically every cell begins to show the rudiments of crystals, that may vary from prismatic to conglomerate in shape. Only along the lateral lines of the perixylary cambium are the crystals extremely small or wholly absent.

Here the perixylary cambium is highly conspicuous. The activity of its lateral stretches is greatly less than in other genera studied. The reason for this is seen in the unique mode of secondary

growth. The internal phloem is usually developed. Occasionally a smaller one of the five outer bundles may be largely or wholly devoid of it. In slightly older stem sections, such as the seventh internode from the apex, a striking new formation can be observed in its incipient stage. Immediately external to two to four of the hard bast patches, the zone of pericambial cells starts division. By the time this phloem tissue is about seven layers deep, its most external layers begin to show lignified thickening, and constitute a hard bast to each of these incipient "extrafascicular" bundles, as the writer proposes to designate them.

In still older sections the zone of pericambial cells outside the hard bast of each normal bundle has indirectly added a certain amount of xylem between itself and the normal hard bast. This apparently is due to the formation of a separate layer of cambial cells, that possibly may represent the inner zone of the extrafascicular bundle phloem tissue, or a special bundle cambium separated from the pericambium.

Following the growth of these bundles into older parts of the stem, as many as ten extrafascicular bundles, one beyond or external to each bundle, may have arisen (fig. 18). As in other genera, the most exaggerated development that the writer has been able to follow lies in the hypocotyl. Here there seems to be a correlating and great distributing center alike for crude and for elaborated sap. While all of the normal bundles in this section showed an extrafascicular bundle, that figured (fig. 18) greatly excelled the other six in the hypocotyl of the plant. The bundle itself lies external to a mass of normal hard bast, that here has split into three now separated patches. Immediately external to the normal hard bast is a zone, that at present can only be called a proliferated pericambium. Beyond this is an extensive plate of xylem traversed radially by two primary extrafascicular bundle medullary rays, and by one or two similar secondary ones. This woody tissue resembles that of the normal bundles; however, as seems to be true of all secondary bundles, the xylem is here wholly devoid of spiral vessels. External to the wood is a zone of five or six thin walled cells, with deeply stained nuclei that together represent the extrafascicular cambium and cambioid layers. Beyond this is

the first formed phloem tissue of these bundles, recognizable in its two parts, an inner or soft bast with large sieve tubes and companion cells, and an outer and now somewhat split hard bast of two or three irregular layers.

In the outer part of the bundle an additional patch of phloem has arisen immediately external to the hard bast just noted. This as yet consists only of soft bast, made up mainly of phloem cells, but with some distinct indications of sieve tubes. Differentiation of two of the outer of these into an additional hard bast has been observed. Finally, an even greater advance than the last is seen in one extrafascicular bundle, where a third soft bast has originated outside the hard bast of the second phloem zone. The areas encountered from without inward to the pith tissue are as follows: (1) epidermis; (2) cortex of large soft cells, two or three layers deep; (3) third or most external soft bast of the extrafascicular bundle; (4) second or most external hard bast of the extrafascicular bundle; (5) second or median soft bast of the extrafascicular bundle; (6) first or most internal hard bast of the extrafascicular bundle; (7) first or most internal soft bast of the extrafascicular bundle; (8) cambium and cambioid tissue of the extrafascicular bundle; (9) xylem (traversed by medullary rays) of the extrafascicular bundle; (10) one to three layers of thin walled cells, endodermis-pericambium (?); (11) hard bast of the normal bundle; (12) soft bast of the normal bundle; (13) intrafascicular cambium and cambioid tissue of the normal bundle; (14) metaxylem of normal bundle; (15) protoxylem of normal bundle; (16) prefascicular cambium (internal section of perixylary cambium); (17) internal phloem; (18) pith.

The writer believes that plants of this species, if vigorously grown and fully nourished, would show an even more evolved formation of tissues than anything described in this paper. He hopes to carry out such studies in the near future.

The crystals already mentioned in their earlier formation have now reached a climax of size and abundance. They show every possible transition, from single tabular forms with straight faces to complex conglomerate types with numerous facets and points.

In the allied species, *Momordica charantia*, an exactly similar formation of extrafascicular bundles takes place, but so far as

observed, they are both fewer in number and more limited in growth capacity, for seldom are there more than two or three of these bundles formed around the stem. More detailed study would be advisable here.

The two species of *Momordica* studied in comparison with all of the other types suggests that this genus has early diverged from some more primitive group which had already developed bicollateral bundles, but had not reached the stage where some of the more complex phloem formations had started. The production, therefore, of a gradually increasing number of extrafascicular bundles that could simulate in their activities the medullary and intraxylary systems, as well as the endocyclic and ectocyclic phloems as auxiliary distributors of elaborated sap, is not without significance. In all of the material available, however, these extrafascicular bundles do not seem to pass higher than the third to seventh epicotylary internode, depending upon the age of the plants.

6. Cucurbita Pepo

The earlier stages of growth conform very closely to *Citrullus*. The later stages, and particularly the structure and growth of the mature hypocotyl, deserve some consideration. When one thinks of the huge fruits borne by the vines of *Cucurbita*, which are matured in six to eight weeks from the time of flowering, it is not surprising that in stems about a month old the phloem often exceeds the xylem in total amount, especially in the large inner bundles, in the ratio of 3:2. While this ratio is diminished later, it still remains true that the total amount of phloem tissue equals or very nearly equals the xylem.

In the lower internodes of fairly mature stems (ten to twelve weeks old), the bundles consist of loose xylem tissue that is deeply divided by secondary, tertiary, and quaternary medullary rays, and the vessels become abundantly filled by intruding tylosis cells, but two features only need be emphasized here. (1) The perixylary cambium, as in other types, in the lower part of the hypocotyl quickly started to form medullary phloem, in part in patches separated by admedullary rays, along the more internal lateral faces of the bundles, and in lines of phloem along the more

external radial faces. The process continued until, in the hypocotyl of stems about four months old, there may be considerable masses of xylem on the inner sides of these, and so, not infrequently, one may count five or six complete collateral transmedullary bundles toward the pith, along with eight to ten purely phloem masses, more externally placed. (2) The internal phloem masses have undergone marked development, especially in the lower part of the axis. In the hypocotyl this is so pronounced that in old stems huge oval phloem areas may project into the broken pith cavity. Each of these, moreover, consists of a wide tract of most internal phloem, of a considerable tract of xylem or, instead, of two or three xylem masses separated from one another by narrow medullary rays; while the most external part of the bundle so narrows toward the protoxylem of each normal bundle that one may well speak here of complete pith bundles. It should be said, however, that for these, as for the extrafascicular bundles of *Momordica*, no spiral tracheae representing a protoxylem exist.

So far as the writer's material has been investigated, these complete pith bundles in *Cucurbita* are the largest yet observed, and deserve more careful and detailed study than has yet been given them.

Physiological inquiry

The extensive development of metaxylem in the root is correlated with the important functional work of carrying upward copious supplies of crude sap from the soil. The development of normal external phloem only in this part of the axis indicates that sufficient elaborated sap can thus be passed down and distributed to even the finest rootlets and root hairs by this tissue, unaided by auxiliary phloem tissues.

The evolution of an internal phloem primarily in the lower part of the young hypocotyl indicates that additional and abundant interchange of elaborated food must be perfectly insured from the region of the hypocotyl upward. In primitive, slow-growing cucurbits, the distribution of elaborated food evidently conforms to that seen in average shrubs and trees. With increasing rapidity of growth and increase of leaf surface, however, new methods have evolved for insuring rapid distribution of the elaborated sap.

The low branching habit of most herbaceous cucurbits, in which three to eight radiating branches start from as many originally young nodes near the ground, necessitates the development of a central correlated system of food exchange that shall reach every part of the aerial axes; hence the extremely complex intercommunications in later stages of growth of the hypocotyl and succeeding epicotylary internodes, so that a balanced distribution of crude sap supply is insured throughout the entire vascular system, upward and downward. The opinion might be ventured that the gradual development of ectocyclic phloem anastomoses may have started as a conduit system for the absorption and transfer of elaborated food gathered from the extensive chlorenchyma of the stem itself. In connection with this, the usually small and abundant (*Momordica*), as well as the few and heavy fruits (*Citrullus*), necessitate rapid and abundant passage of elaborated sap to these, no matter whether situated along a main axis or some side branch.

As in the case of previous investigators on the Cucurbitaceae, the writer has frequently referred to the marked reduction in size, or the occasional absorption of one, two, or rarely three bundles in the normal ring. It seems probable that such may be due to mechanical strains operating along the axis, and also dorsiventral light relation, for it should be remembered that while the rapidly growing cucurbits are provided with tendrils, and show many other characteristics of climbing plants, they are usually grown as scrambling vines that exhibit marked dorsiventrality after two or three weeks of growth.

The writer has been impressed by the capacity and the plasticity or adaptability shown by different areas of parenchyma for taking on new tissue formation. TAYLOR (**18**) demonstrated the transformation of prospective xylem cells into phloem, or vice versa, and the formation of bundle in pith cells under appropriate stimuli. Similarly, in the gradual evolution of the herbaceous, and particularly of the rapidly growing and succulent herbaceous cucurbits, it seems that any parenchyma or tissue center within the endodermis, possibly even beyond (cf. FISCHER'S ectocyclic sieve tubes), is capable of modifying its future tissue history.

From the standpoint of plant mechanics, attention might now be drawn to a curious tissue change noted in several genera (*Cucurbita*, *Momordica*, *Benincasa*, and *Lagenaria*). In all of these the young roots and hypocotyl early develop strong patches of hard bast along the exterior of each bundle. These collectively seem to give a degree of tensile strength and resistance that would be eminently helpful against tugging strains exerted on the hypocotyl and root. As one approaches the cotyledonary junction, these thin out and disappear, but as the epicotyl lengthens a lignified endocortex early arises and persists for several weeks, although it gradually becomes stretched and broken through the growth expansion of the stem. Such an expanded cylinder must act as a pliable and adjustable strengthening zone in the resistance to wind strains and torsions. On the other hand, in *Momordica*, the hard bast patches of the root are continued upward through the stem, and, together with the extrafascicular bundles, provide sufficient support, and in such a case no fibrous endocortex is encountered.

Summary

1. Members of this group may form a growth of stem and leafage within the growing period of four months that is probably unequaled by any other annual herbaceous plant family. *Cucurbita Pepo* may show a stem and branch length together totaling over 140 feet, producing probably as many as 450 leaves. Plants propagated at the same time may show a surprising total growth regularity. Three specimens, differing greatly in number of branches and general external habit of growth, when measured totaled 37.25, 37, and 39 feet, and produced respectively 120, 123, and 123 leaves.

2. For a proper estimation of the nutritive activities of these plants detailed investigation must be made of the structure and growth of all parts of the plant axis, from the seedling to the mature state.

3. Study of four representative seedlings reveals in all a tetrarch bundle system in the root, each radial arm of which undergoes splitting of the protoxylem and passage of the protoxylem halves toward the metaxylem, and later uniting in front of it to constitute the four protoxylem areas of the four stem meristeles.

4. The tetrarch vascular system in the root separates into four meristeles at the base of the seedling hypocotyl. These, in their turn, start to split radially until eight or ten bundles are formed, which traverse the greater length of the hypocotyl.

5. In this transition, the four meristeles that move apart as the pith expands in the base of the hypocotyl show no trace of internal cambium. This remains true for the lowest 2–5 mm. of the hypocotyl. In their earliest seedling condition, therefore, these bundles are only collateral, in this respect resembling the more primitive shrubby cucurbits.

6. About 2–3 mm. above the summit of the root, the intrafascicular cambium of each bundle is seen to become continuous from outside inward, with a zone of fundamental cells around the lateral and anterior faces of each bundle, and thus originates what the writer designated "perixylary" cambium.

7. Through immediate activity of the inner cells of this cambium ("prefascicular" cambium), patches of internal phloem originate in front of each bundle.

8. In all of the seedlings studied, the meristeles constitute a ring of four, in time becoming eight or ten members, four or five of the ten being slightly larger and more internal, the rest slightly smaller and more external. The whole constitutes a typical dicotyledonous meristele system. There is no ground for the view that the Cucurbitaceae showed primitively multiseriate or irregularly scattered bundle disposition.

9. In transition from the hypocotyl to the epicotyl, similar details of structure and growth are usually shown. To this there is an exception, for some cucurbits develop only eight or ten strong hard bast areas, which are directly continued into the epicotyl (*Momordica*), while in most the hard bast of the radicle and hypocotyl thins out and disappears, and a lignified endocortex ring becomes the substitute layer. The writer accepts it that the early formed fibrous zone is endocortex. Within it, a one-layered endodermis and one to three-layered pericambium originate.

10. Growth of the internal phloem is effected by activity of the prefascicular cambial section, although for a time the lateral pericambial sections remain dormant. Simultaneously with the

last, sinuous interfascicular cambial patches join with the intrafascicular to form a continuous and typically dicotyledonous cambium ring. A marked difference is observable between the small flat cambial cells of the latter and the large clear cells of the former.

11. Contemporaneous with both of these, continued division and growth of pericambial cells gradually cause stretching of the exterior part of the stem, and rupture of the fibrous endocortex where such exists.

12. Great variation is shown in the amount and degree of lignification of the developing xylem in different types, from highly parenchymatous bundles of *Citrullus* and *Cucurbita* to the dense and fibrous types such as *Luffa* and *Momordica*.

13. Tylosis cells invading the pitted vessels are observed in practically all of the genera in varying extent.

14. Crystals are very abundant and highly visible in the medullary rays and pith cells in *Momordica*.

15. The reactivated perixylary lateral cambia give rise to varying amount of lateral phloem: (1) scarcely traceable in *Momordica*, (2) slightly in *Trichosanthes*, (3) well marked as platelike patches in *Luffa*, (4) extensive in *Lagenaria*, and (5) most pronounced in *Citrullus*.

16. Such lateral medullary phloem becomes, with the addition of metaxylem, "transmedullary" bundles which attain their greatest size and variety of structure in the mature hypocotyl.

17. These masses of phloem develop from the perixylary cambial segments tangentially.

18. In the formation of these transmedullary bundles, "admedullary" rays separate the inner and larger ones.

19. Endocyclic sieve tube connections, as traced by FISCHER, become increasingly extensive with age, especially in the larger and softer types.

20. Soon after the formation of the endocyclic, ectocyclic phloem is formed by outpushing of the phloem between the burst fibrous endocortex patches, and so invasion of the mediocortex results.

21. The internal phloem areas may remain rounded and sharply demarcated as projections into the pith in the stem, but in the

lower part of the axis and in the hypocotyl of the more rank growing types these may enlarge greatly, becoming attenuate toward the protoxylem front, and eventually become occluded into the pith, after developing centrifugal xylem, as "obcollateral" bundles.

22. More or less throughout the older axis, and especially in the hypocotyl of *Cucurbita Pepo* and *Citrullus vulgaris*, "intraxylary" phloem in the xylem parenchyma appears.

23. An extensive system of secondary medullary rays arises in all types, the manner and depth of these corresponding to the thickness of the stem.

24. In species of *Momordica* alone a unique formation of "extrafascicular" bundles is observed, and these do not develop simultaneously and are not of equal size, but in succession over the stem. They extend from the hypocotyl only through a limited number of nodes of the epicotyl, to disappear above.

25. The xylem and phloem bridge connections link all the meristeles together, especially in the hypocotyl.

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LITERATURE CITED

1. BARANETSKY, J., Recherches sur les faisceaux bicollateraux. Ann. Sci. Nat. Bot. VIII. 12:301–304. 1900.
2. DEBARY, A., Comparative anatomy of the vegetative organs of the phanerogams and ferns. (Eng. trans.). 1884.
3. FISCHER, A., Untersuchungen über die Siebröhrensystem der Cucurbitaceen. Berlin. 1884.
4. GERARD, R., Passage de la racine a la tige. Ann. Sci. Nat. Bot. VI. 11:279–426. 1881.
5. HANSTEIN, J., Die Milchsaftgefässe und die verwandten Organe der Rinde. Berlin. 1864.

6. HARTIG, T., Über die Querscheidewände zwischen den einzelnen Gleidern der Siebröhren in *Cucurbita Pepo*. Bot. Zeit. 51–54. 1854.
7. HERAIL, J., Étude de la tige des Dicotyledones. Ann. Sci. Nat. Bot. VII. **2**:201–310. 1885.
8. LAMOUNETTE, B., Recherches sur l'origine morphologique du liber interne. Ann. Sci. Nat. Bot. VII. **11**:194–278. 1890.
9. PETERSEN, O. G., Über das Auftreten bicollateraler Gefässbündel in verschiedenen Pflanzenfamilien und über den Werth derselben für die Systematik. Engler's Jahrbücher **3**:359–402. 1882.
10. PITARD, J., Sur les faisceaux Liberiens tertiares des tiges des Cucurbitacees. Actes Soc. Linn. Bordeaux **66**:104–105. 1901.
11. POTTER, M. C., On the increase in thickness of the stem of the Cucurbitaceae. Proc. Camb. Phil. Soc. **7**:14–15. 1889.
12. ———, Additional note on the thickening of the stem of the Cucurbitaceae. Proc. Camb. Phil. Soc. **7**:65–67. 1890.
13. RUSSOW, E., Betrachtungen über das Leitbundel und Grundgewebe. Dorpat. 1875.
14. SCOTT, D. H., and BREBNER, G., On internal phloem in the root and stem of dicotyledons. Ann. Botany **5**:259–297. 1891.
15. SCHRIEBER, F., Das Dickenwachstum des monocotyledonischen und dicotyledonischen Stammes nach Schacht mit Rücksicht auf NÄGLEI. Bot. Zeit. 367–372. 1865.
16. SOLEREDER, H., Über den systematischen Werth der Holzstructur bei den Dicotyledonen. Munich. 1885.
17. ———, Systematic anatomy of the dicotyledons. **1**:389–397. 1908.
18. TAYLOR, W. R., On the production of new cell formations in plants. Contrib. Bot. Lab. Univ. Penn. **4**:271–294. 1919.
19. THOMPSON, C. B., The structure and development of internal phloem in *Gelsemium sempervirens*. Contrib. Bot. Lab. Univ. Penn. **2**:41–53. 1904.
20. TONDERA, F., Über den sympodialen Bau des Stengels von *Sicyos angulata*. Sitz. Math.-Natur. Cl. Kais. Akad. Wissen. **111**:317–326. 1902.
21. VESQUE, J., Anatomie comparee de l'ecorce. Ann. Sci. Nat. Bot. VI. **2**:82–197. 1875.
22. VON FABER, F. C., Zur Entwicklungsgeschichte der bicollateralen Gefässbündel von *Cucurbita Pepo*. Ber. Deutsch. Bot. Gesell. **22**:296–304. 1904.
23. VON MOHL, H., Einige Andeutungen über den Bau des Bastes. Bot. Zeit. 873–881. 1855.
24. WEISS, J. E., Das markständige Gefässbündelsystem einiger Dikotyledonen in seiner Beziehung zu den Blattspuren. Bot. Centralbl. XV. **3**:318–327. 1883.
25. WORSDELL, W. C., The origin and meaning of medullary (intraxylary) phloem in the stems of dicotyledons. Ann. Botany **29**:567–590. 1915.
26. ZIMMERMANN, A., Die Cucurbitaceen. 1. Jena. 1922.

EXPLANATION OF PLATES I–III

Abbreviations: *cex*, cambium of extrafascicular bundle; *ec*, external or intrafascicular cambium; *ecst*, ectocyclic sieve tubes; *enc*, endocortex; *enst*, entocyclic sieve tubes; *eph*, external phloem; *hb*, hard bast; *hbex*, hard bast of extrafascicular bundle; *ic*, internal or prefascicular cambium; *ifc*, interfascicular cambium; *iph*, internal phloem; *ixph*, intraxylary phloem; *mc*, medullary (ray) cambium; *mph*, medullary phloem; *p*, pith; *phco*, phloem bridge connection; *pmr*, primary medullary ray; *pmrex*, primary medullary ray of extrafascicular bundle; *pv*, pitted vessel; *rph*, rudimentary phloem; *sb*, soft bast; *sbex*, soft bast of extrafascicular bundle; *smr*, secondary medullary ray; *smrex*, secondary medullary ray of extrafascicular bundle; *sv*, spiral vessel; *tmr*, tertiary medullary ray; *xco*, xylem bridge connection; *xex*, xylem of extrafascicular bundle; *xp*, xylem parenchyma.

PLATE I

FIG. 1.—Diagrammatic transverse section of seedling root of *Luffa aegyptiaca*, showing typical tetrarch monostele; ×90.

FIG. 2.—Diagrammatic transverse section of seedling root of *Luffa aegyptiaca:* monostele splitting into four meristeles, two xylem masses larger, two smaller; note development of side root in line with a xylem arm; ×90.

FIGS. 3–6.—Stages in separation of root monostele into four stem meristeles: ×150.

PLATE II

FIG. 7.—One of meristeles represented in fig. 6, showing development inward of perixylary cambium forming internal or prefascicular cambium; note spiral tracheae at both extremities of bundle; ×250.

FIG. 8.—Same, showing further development of internal or prefascicular cambium, here given rise to small patch of internal phloem; note moving inward of spiral tracheae to position immediately internal to pitted vessels; ×250.

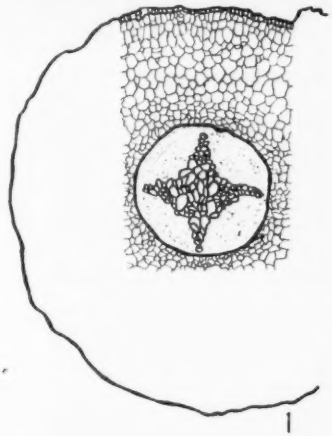
FIG. 9.—Transverse section of meristele of *Luffa aegyptiaca* in lower hypocotyl just above level of "heel," showing perixylary cambium and newly developed internal phloem; ×160.

FIGS. 10, 11.—Transition stages from root to hypocotyl in seedling of *Momordica balsamina;* note hard bast already developed; ×115.

FIG. 12.—Transverse section of meristele in lowest part of seedling hypocotyl of *Momordica balsamina*, showing prominent hard bast; ×115.

PLATE III

FIG. 13.—Diagrammatic transverse section of mature stem of *Trichosanthes colubrina*, fifth internode from apex, showing broken sclerenchyma ring (*enc*), rudimentary phloem areas (*rph*) of two inner bundles; note commencing medullary phloem (*mph*) (interfascicular cambium not shown); ×15.



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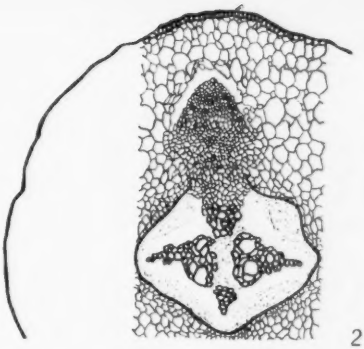

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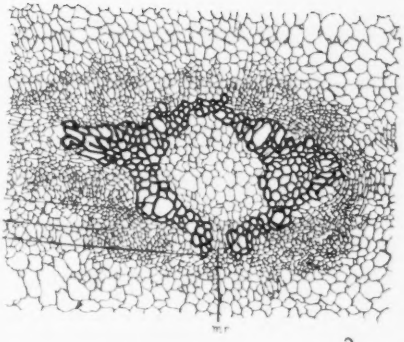

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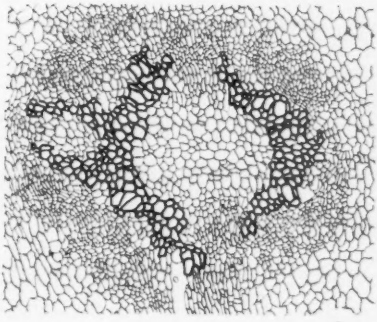

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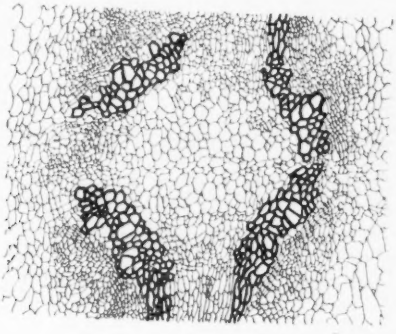

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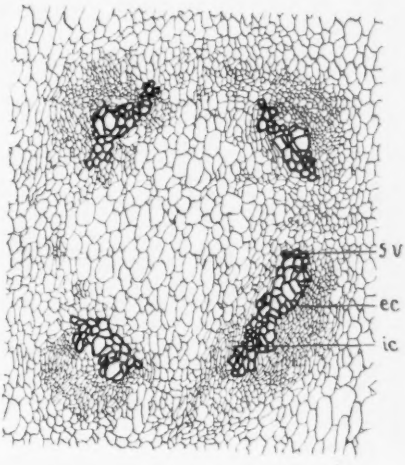

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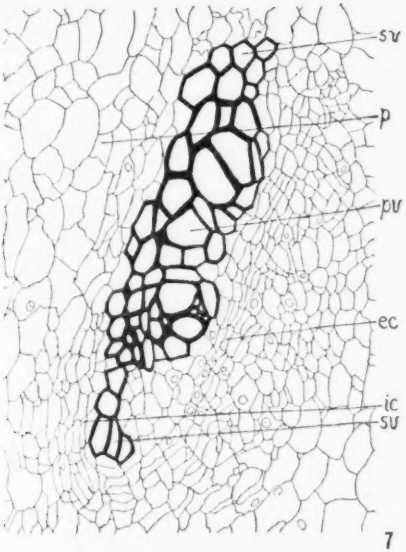
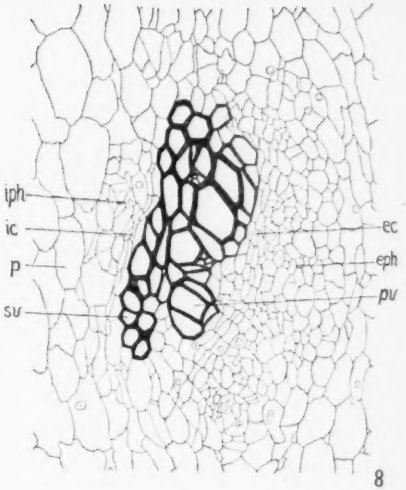
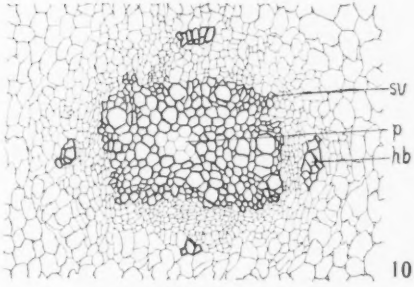
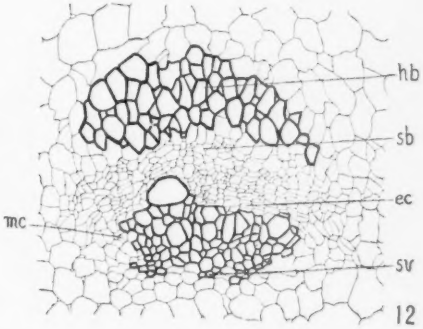
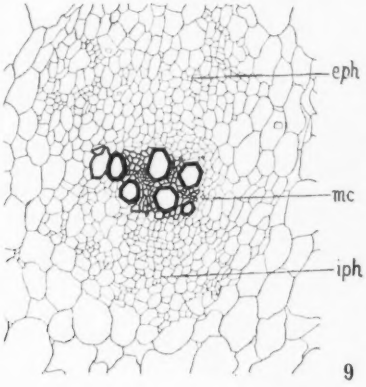
HOLROYD on CUCURBITACEAE

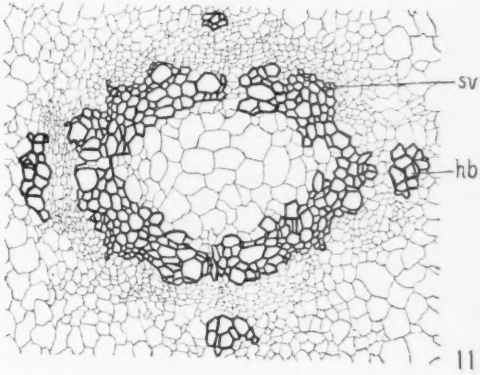




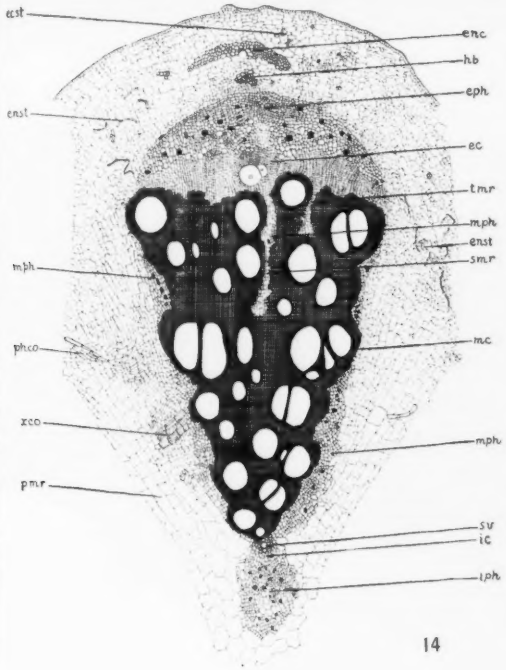








HOLROYD on CUCURBITACEAE



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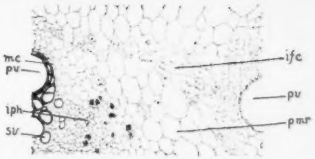

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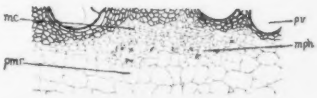

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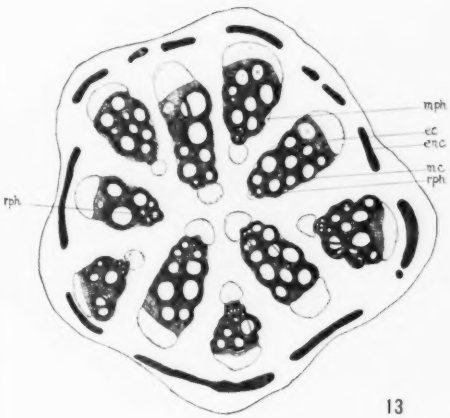

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HOLROYD on CUCURBITACEAE

FIG. 14.—Same, first epicotylary internode above hypocotyl, showing medullary phloem in primary and secondary medullary rays (*mph*), xylem and phloem bridge connections (*xco*, *phco*), ectocyclic and endocyclic sieve tubes (*ecst*, *enst*); (in part diagrammatic); ×25.

FIG. 15.—Transverse section of medullary ray of *Luffa aegyptiaca*, showing conspicuous interfascicular cambium in stages of development; ×32.

FIG. 16.—Transverse section of mature hypocotyl of *Luffa aegyptiaca*, showing section of primary medullary ray with medullary phloem and part of perixylary cambium extending alongside metaxylem; ×32.

FIG. 17.—Transverse section of xylem area of mature hypocotyl of *Citrullus vulgaris* at end of growing season, showing intraxylary phloem in xylem parenchyma; note tylosis cells in pitted vessels; (in part diagrammatic); ×25.

FIG. 18.—Diagrammatic transverse section of mature hypocotyl of *Momordica balsamina*, showing details of extrafascicular bundles adjacent to each normal bundle; note persistent hard bast of normal bundles, one to three alternating layers of hard and soft bast of extrafascicular bundles, absence of medullary and intraxylary phloem, and absence of sclerenchymatous endocortex; ×15.

DIVERGENCE OF CATALASE AND RESPIRATION IN GERMINATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 320

LOUISA E. RHINE

(WITH FOUR FIGURES)

Introduction

Interest in catalase centers around two main points: (1) its nature and function, and (2) its use as a measure of respiratory or metabolic activity. Little is known of its nature except that it acts like an enzyme in hastening a reaction that would proceed very slowly without it. Hydrogen peroxide decomposes spontaneously, but very slowly. The single known property of catalase is its power to hasten greatly this reaction. It is further like an enzyme in its temperature relations (**24, 29, 34, 40**), and in the general methods by which it may be extracted and precipitated. Although such facts as these have led more and more to the general belief that catalase is an enzyme, as the earlier workers (**25, 29, 37**) thought, it is for convenience rather than certainty that it is so spoken of here. Although the knowledge of the nature of catalase is far from complete, its function in the cell is still more of a mystery. By far the majority of workers in this line have found some connection between catalase and oxidation or respiration, but the data reported offer too many contradictions to permit of any conclusions regarding the nature of this connection. Among the various theories advanced regarding the possible function of catalase in the cell is that of BURGE and his co-workers (**8, 10, 11, 12, 13, 14**). This interprets catalase as having a causal relation to oxidation, apparently because catalase activity and oxidation rate parallel each other in many cases, from which the conclusion is drawn that the one is the cause of the other. This theory might be taken more seriously if it were the only possible explanation of the facts, and if there were not so many instances in which catalase activity and

respiration are not parallel. Even though in many cases a high metabolic condition in tissue is accompanied by a high catalase content, the accumulation of evidence showing discrepancies in the two processes (**1, 8, 33, 39, 41, 44, 46**) should be taken as indication that this parallelism may be incidental rather than fundamental.

Although numerous other theories of the function of catalase have been advanced (**6, 7, 20, 21, 25, 45**), that of LOEW (**29**) has perhaps been most widely accepted (**23, 30, 38**). This rests on the supposition that hydrogen peroxide is formed in the process of oxidation, and that catalase functions to protect the cell against toxic concentrations of it by decomposing it at once into harmless constituents. The chief objection has been that hydrogen peroxide has never yet been demonstrated as actually occurring in cell oxidations, although it seems not improbable that it is so formed (**17**). Besides this, catalase apparently will not react with other peroxides (**5**). As LOEW (**30**) points out, however, the fact that the presence of hydrogen peroxide has not been proved is not so great an objection to the theory as it might seem, since even if it were formed in a cell containing catalase it would necessarily be decomposed almost instantaneously and before it could be detected experimentally. Besides this there is the other supposition, that, although no other substance except hydrogen peroxide has been found on which catalase will act in vitro, it is quite possible that it functions in the cell as LOEW suggested, but upon an unknown substance which may equally well be formed in the process of oxidation.

The fact that in so many cases (**2, 3, 4, 10, 12, 13, 14, 16, 19, 28, 31, 46, 47**) a parallelism has been shown between catalase and respiration has led in some degree to the use of catalase as a measure of oxidation rate. If it could be demonstrated that this is a safe procedure, it would certainly offer a convenient substitute for the tedious and too often impossible direct measurement of respiration and metabolic rate. The discrepancies mentioned, however, indicate that more must be known of the nature of catalase and its place in metabolism before it can be decided how far its use as an indicator is safe and valid. In the paper here presented it is hoped to shed some light upon this problem.

Method of catalase determinations

The method used was essentially that described by APPLEMAN (**2**), in which the oxygen liberated is measured by a manometer with adjustable arm, so that excess pressure during the reaction is avoided. The water bath was kept between 21° and 22° C. and all results calculated to standard conditions of temperature and pressure. Five cc. of Oakland "dioxogen" neutralized with N/10 NaOH was used in all cases. The tissue was ground in a mortar with a small quantity of sand and an excess of $CaCO_3$. It was washed quantitatively into the shaking bottle with about 15 cc. of distilled water. When it had reached the temperature of the bath, the hydrogen peroxide was added 15 seconds before the shaking began. One 10-minute reading was taken.

In using various kinds of seeds in different conditions of moisture content, it was found impossible to adjust the degree of pulverization of tissue by any arbitrary method. Since the determinations were to be quantitative, a sieve could not be used, and it was equally impossible to choose a single length of time of grinding for all seeds, owing to the great diversity of hardness, moisture content, and general character of the tissue. The material was ground, therefore, to what in best judgment seemed to be a comparable state of fineness and homogeneity with that attained in grinding dry wheat four minutes.

MORGULIS and others (**9, 32, 33**) object that a time reading, arbitrarily chosen for the measurement of the catalase-hydrogen peroxide reaction, cannot afford an accurate measurement of catalase activity. Under the law of mass action, an arbitrary time limit would not express the results of comparable stages in the reaction when the amount of one or more substances was varied. Thus when the proportion of catalase to hydrogen peroxide is high, a 10-minute reading would express the results of a much greater part of the entire reaction than it would when the amount of catalase is relatively small. The data in table I will make this clear. The reactions were allowed to run to completion, one, two, five, ten minute and final readings made, and the time until the end of the reaction recorded. Dry wheat was the tissue used.

It is apparent from table I that a 10-minute reading does not give a measure of the maximum amount of oxygen delivery in any but one of the tests made. Five cc. of "dioxogen" is capable of yielding 67.8 cc. of oxygen, as measured by the maximum amount secured with an excess of catalase-containing tissue. In every case except the first two, therefore, the amount of catalase present was in excess of the peroxide. The greater this excess, the shorter is the time required to complete the reaction.

MORGULIS (**32**) has shown further that the presence of a great excess of either hydrogen peroxide or catalase exerts a depressing influence on the reaction. This is shown in table I in every case beyond the second. MORGULIS criticizes the "entire method of

TABLE I

No. of grains	Weight in gm.	O_2 (cc.) liberated in				Final amount of O_2 (cc.)	Total time in minutes
		1 minute	2 minutes	5 minutes	10 minutes		
5	0.185	3	5	8	12	27.3	120
10	0.385	6	11	18	26	60.8	105
15	0.001	10	16	30	40	67.8	105
25	0.033	12	20	33	45	67.1	55
50	1.844	22	34	51	56	.....	.....
75	2.806	23	37	57	66	67.8	25
150	5.2	30	40	63	67	67.2	14

comparing several samples of catalase on the basis of the amount of oxygen which they respectively liberate," and states that the comparison should rather be "between respective quantities of catalase preparation required to set free the same amount of oxygen from a given quantity of peroxide." This change in method, however, would involve a great loss of time in determining the correct amount of catalase to be used at each determination, and is unnecessary because the whole error involved in the old method can be corrected for with comparative ease, by a simple set of factors designed to apply within a wide range of possible oxygen delivery. The following set has been planned to apply to the kind of material and reaction that has been used in the following experiments. Dry wheat was pulverized, sifted through a 100-mesh screen, and thoroughly mixed to give a flour of the greatest possible homo-

geneity. Catalase determinations were then made on amounts of flour varying from 0.02 to 2 gm., at close intervals. A 10-minute reading was taken, and from this and the weight of material used the catalase activity per gram was calculated. Table II shows the results and the factors calculated from the maximum value per gram.

These results indicate that when the amount of catalase present during a determination is so adjusted that it liberates about 19 cc. of oxygen in 10 minutes, the maximum value is being obtained. Thus it follows that when using 5 cc. of full strength neutralized "dioxogen" at 21°–22° C. with pulverized seed material, results

TABLE II

Weight in gm.	O_2 (cc.) liberated in 10 minutes	O_2 (cc.) per gm.	Factor
0.02	0.491	24.55	1.34
0.04	1.01	25.55	1.30
0.06	1.56	26.00	1.26
0.08	2.06	25.76	1.28
0.1	3.01	30.10	1.07
0.2	6.38	31.94	1.03
0.4	12.92	32.30	1.02
0.6	19.77	32.95	1.00
0.8	24.55	30.69	1.06
1.0	30.80	30.80	1.07
1.2	35.82	29.84	1.13
1.4	39.40	28.20	1.14
1.6	42.02	26.26	1.25
1.8	44.49	24.71	1.33
2.0	47.82	23.91	1.37

will be true when the oxygen liberated in 10 minutes is about 18–20 cc. Results on either side of this figure can be corrected for by using the factors given in column four. This set of factors, however, cannot be used to give accurate correction for all types of tissue that might be investigated, for it seems evident that the bulk of material present in the comparatively inert dry seed meal exerts some influence on the course of the reaction. Table III shows data similar to those given in table II, but in this case, instead of the wheat flour, a comparatively active water extract of young rye ovules was used.

Fig. 1 shows the data presented in tables II and III in the form of curves. While the same general type of curve is secured in each

instance, there is a distinct difference in the rate of depression and in the position of the highest value in each curve. Since the material used in all of the experimental work which follows was of the ground seed type represented in table II and curve I of fig. 1 the corrections indicated in table II have been used throughout.

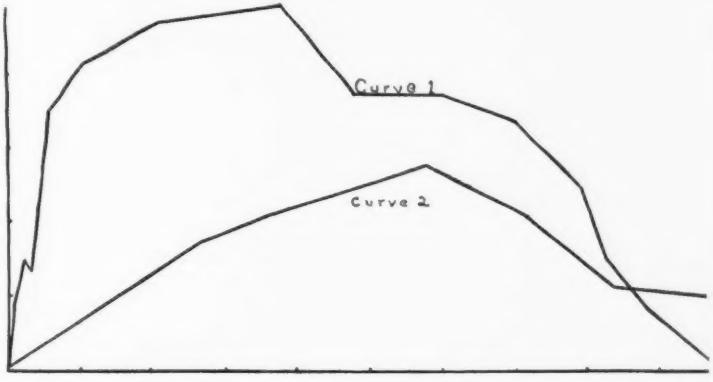

FIG. 1.—Curves showing variation in type of reaction of two kinds of catalase-containing tissue: horizontal axes represent cc. of O_2 delivery in 10 minutes, vertical axes represent cc. of O_2 delivery per unit; curve I, from data in table II (O_2 from 1 gm. taken as unit); curve II, from table III (O_2 from 3 cc. taken as unit).

TABLE III

Extract (cc.)	O_2 (cc.) liberated in 10 minutes	O_2 (cc.) per cc.	Factor
0.5	4.0	8.0	1.18
1.0	8.65	8.65	1.09
1.5	13.32	8.96	1.05
2.0	18.66	9.33	1.01
3.0	28.43	9.47	1.00
4.0	36.26	9.05	1.04
5.0	43.48	8.60	1.10
6.0	50.15	8.35	1.14

It may be noted in passing, however, that in no case have the corrections involved been sufficiently large to change the nature of the result in a radical degree. An attempt was always made to adjust proportions somewhat to secure results ranging as closely as

possible around 18–20 cc. of oxygen delivery. Since the great individual variation in seeds makes it impossible to secure perfect checks, all determinations were run in triplicate, and the numbers reported are the averages of these determinations.

All the seeds used were obtained from Vaughan and Company, Chicago, and were of the 1922 crop. Only those showing a germination of 95 per cent or better were used.

TABLE IV

WHEAT, 10 SEEDS PER TEST, WEIGHT 0.385 GM.

Time	Condition	Uncorrected O_2 (cc.)	Factor	Corrected O_2 (cc.)
	A. Germination temperature 10°–12° C.			
Days				
0	Dry	21.50	1.00	21.5
1	Germination	11.53	1.02	11.76
2	Germination	10.15	1.02	10.36
3	Germination	10.16	1.02	10.36
4	Germination	10.43	1.02	10.64
5	Germination	11.09	1.02	11.31
6	Germination	12.64	1.02	12.89
7	Germination	17.47	1.00	17.47
8	Germination	17.80	1.00	17.80
	B. Germination temperature 23°–26° C.			
Hours	Dry	21.50	1.00	21.50
4	Germination	13.98	1.02	14.25
11	Germination	12.75	1.02	13.00
15	Germination	14.47	1.02	14.75
24	Germination	20.77	1.00	20.77
41	Germination	36.05	1.13	40.74

Investigation

A study of the relationship between catalase activity and respiration rate under conditions of rapid change was undertaken in the hope that it would throw some light upon the nature of catalase and its relation to oxidation. Germination and ripening of seeds seemed to offer examples of such change. These phases were therefore chosen for detailed study.

CATALASE IN GERMINATING SEEDS.—Tables IV–IX show the results of catalase determinations made upon six different kinds of seeds at various stages in their germination. For convenience of

determination, and to avoid somewhat the error introduced from individual variations in seeds, small seed types were chosen, and ten or more seeds used in each test according to their relative

TABLE V

FETERITA, 10 SEEDS, WEIGHT 0.332 GM. 23°–26° C.

Hours	Condition	Corrected O_2 (cc.)
0	Dry	13.0
2	Germination	8.14
7	Germination	6.19
14	Germination	6.52
21	Germination	7.19
30	Germination	9.5
40	Germination	10.06
48	Germination	16.69

TABLE VI

CLOVER, 10 SEEDS, WEIGHT 0.007 GM. 23°–26° C.

Hours	Condition	Corrected O_2 (cc.)
0	Germination	18.3
2	Germination	12.77
8	Germination	8.79
22	Germination	8.63
28	Germination	4.09
48	Germination	10.56

TABLE VII

MUSTARD, 280 SEEDS, WEIGHT 2 GM., 25° C.

Hours	Condition	Corrected O_2 (cc.)
0	Dry	3.56
5	Germination	2.88
11	Germination	2.96
24	Germination	12.15
33	Germination	14.19

TABLE VIII

RADISH, 25 SEEDS, WEIGHT 0.2 GM., 25° C.

Hours	Condition	Corrected O_2 (cc.)
0	Dry	26.36
0.5	Germination	21.36
1.0	Germination	20.75
2.0	Germination	24.89
3.0	Germination	31.83

TABLE IX

BUCKWHEAT, 20 SEEDS, WEIGHT 0.515 GM., 25° C.

Hours	Condition	Corrected O_2 (cc.)
0	Dry	13.15
4	Germination	8.15
10	Germination	5.87
18	Germination	4.70
27	Germination	15.07
34	Germination	26.02

activity. The six kinds of seeds studied were selected further to include starchy, fatty, and protein types, but beyond that were chosen at random.

Tables IV–IX and fig. 2 show that at the very outset of germination there is a decided and unmistakable drop in the amount of catalase per seed. In each of the six different kinds of seeds tested the same striking decrease is shown. It begins apparently as soon as the seed is put in germinating conditions, and the period of its duration is proportionate to the rate of germination. The extreme of rapidity of this initial drop is interestingly shown in the data on radish in table VIII. Here in one-half to one hour the lowest point is reached, and in three hours the seed is practically germinated, the testa being ruptured and the cotyledons protruding. In

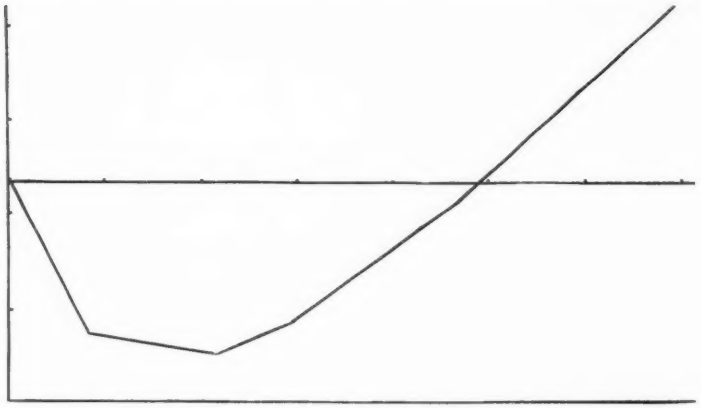

FIG. 2.—Typical catalase germination curve, plotted from data in table IV*A*: horizontal axes represent time of germination in hours; vertical axes represent cc. of O_2 delivered in 10 minutes.

other cases the depression lasts longer. In wheat at 10°–12° C. (table IV*A*) the lowest point recorded is not reached for several days, while in the same seed at 23°–26° C. it is reached in half a day. This difference, however, is quite in proportion to the time required for the germination of wheat at these two temperatures.

This decrease in catalase is quickly followed by a sharp rise, such as has ordinarily been reported for catalase during germination (**18, 26, 36**). The very early stage at which the drop occurs is doubtless why it has been overlooked in previous work, since by the time the hypocotyl is protruded, in most of the seeds tested, the

catalase activity is back again to about the amount that had been present in the dry seed.

CATALASE IN WET SEEDS DEPRIVED OF OXYGEN.—Coincident with the study of the germination of seeds, the effect of prolonged soaking in water was tested.

The data on soaking seeds confirm those secured on the germinating ones. The same initial decrease found in that case is present here also. In this case, however, the curve continues to go down, with no subsequent rise such as was shown in the data on germination. The seeds used in table IV*A* and table X*A* were kept side by side on ice during the same period, so that the two sets can well

TABLE X

WHEAT, 10 SEEDS PER TEST

Days	A. 10°–12° C.		Days	B. 15°–16° C.	
	Condition	Corrected O_2		Condition	Corrected O_2
0	Dry	21.5	0	Dry	17.17
1	Soaking in tap water	15.66	1	Soaking in boiled distilled H_2O	14.95
2	Soaking in tap water	15.88	2	Soaking in boiled distilled H_2O	14.31
3	Soaking in tap water	11.28	3	Soaking in boiled distilled H_2O	12.46
4	Soaking in tap water	12.02	10	Soaking in boiled distilled H_2O	8.89
5	Soaking in tap water	10.52	17	Soaking in boiled distilled H_2O	7.31
7	Soaking in tap water	11.20	22	Soaking in boiled distilled H_2O	0.6
8	Soaking in tap water	11.02	29	Soaking in boiled distilled H_2O	0.5
15	Soaking in tap water	10.98	22	Germination 2 per cent	.....
19	Soaking in tap water	10.44	29	Germination none	.....

be compared. There is significance in the fact that the germinating seeds show a more rapid decrease at first than the soaking ones. This will be discussed later. This experiment was interrupted by accident, but was repeated later in table X*B*. In this case, however, a somewhat higher temperature, and boiled, distilled water (in place of the not entirely oxygen-free tap water) was used, and the time for catalase exhaustion reduced to 22–29 days. At the end of 22 days the seeds were tested for viability, and a feeble germination of about 2 per cent was secured. In 29 days none germinated. A decrease in catalase activity with soaking of seeds has been noted incidentally in earlier work (**35, 41, 42**), but its significance was not recognized.

DRY SEEDS DEPRIVED OF OXYGEN.—Since catalase was seen to decrease to practical exhaustion under oxygen-free conditions in the presence of water, an attempt was made to produce the same result on dry seeds. Wheat grains were imbedded in soft paraffin to exclude the air, and tested again after 65 days. The catalase activity was still practically unchanged, however, and it was evident that changes in the dry seeds resulting from lack of oxygen occur at such a slow rate that a much longer time would have to elapse before they would be measurable.

DISTRIBUTION OF CATALASE IN SEED DURING GERMINATION.—In order to learn something of the distribution of catalase in different parts of the seed in various stages of germination, a series of tests was made. Dry seeds could not be separated into endosperm

TABLE XI

WHEAT, 10 SEEDS WHOLE, ENDOSPERM OR EMBRYO, PER TEST

No. of test	Condition	O_2 whole seed (cc.)	O_2 endosperm (cc.)	O_2 embryo (cc.)
1	Dry	21.5	.....	.....
2	Soaked 1 hour	17.00	13.0	6.0
3	Germination, 2 days 10°–12° C.	10.15	6.7	3.7
4	Germination, 7 days 10°–12° C. (just sprouted)	17.49	7.0	8.0
5	Germination, 41 hours 25° C. (well sprouted)	36.00	15.0	16.0
6	Germination 3 days 25° C. (seedlings)	.....	19.0	.....

and embryo with scutellum, and, therefore, seeds soaked for one hour (table XI, no. 2) had to be used instead to give the closest possible indication of the distribution of catalase in a dry seed. No. 3 of table XI shows the distribution of catalase between embryo and endosperm when the activity of the entire seed is at its lowest ebb in germination. Nos. 4–6 show the distribution as the activity of the whole seed rises. The pertinent facts brought out by table XI are: (1) that both the endosperm and the embryo show a drop in catalase paralleling that in the whole seed; and (2) that the endosperm as well as the embryo shows a later increase. Of course it does not rise as high in the endosperm as in the enlarging embryo, but no. 6 shows it to be higher again than it was in no. 2, at least,

although by this time it appears quite dry and shriveled. It must be remembered, however, that the layer of aleurone cells with their living protoplasm is included in all the tests on endosperm.

RESPIRATION OF SEEDS DURING GERMINATION.—In the past catalase and respiration during germination have always been reported as paralleling each other, and increasing steadily (**15, 18, 26, 36, 41, 46**). Since, however, it is shown that catalase activity decreases in early germination, it becomes important to know what course respiration takes during this period. Either respiration must also suffer a decrease with the onset of germination, or, as has been supposed, it increases steadily from the beginning of germination. If the latter should be the case, we would have here a striking divergence in the two curves. In the literature there are many instances of small differences in the rate or intensity of the two processes, but since apparently there is no case on record showing decrease of catalase activity with increase of respiratory rate, it was thought worth while to make a careful study of the respiration, at short intervals, of germinating seeds throughout the period of catalase decline.

Since no convenient method was available for measuring increase of respiration consecutively on the same seed sample at short intervals, a simple apparatus was contrived by which such data could easily and quickly be secured. The principle involved was the direct measurement of changes in air volume arising from respiration when the carbon dioxide is absorbed by NaOH. By this method a seed sample could be used that was sufficiently large to overcome the error arising from the individual variations of the seeds, and readings could be taken at will on the same seeds throughout the germination period. An outline of the apparatus is shown in fig. 3.

Two instruments thus fitted can be used, one containing the seeds and serving as the test, the other, with everything but the seeds, being a control on temperature and pressure conditions. This obviates the necessity of making calculations for the corrections necessary for these variables, which would have to be made if one instrument were used alone. The temperature of the two vessels must always be kept the same, however, since in air volumes

of this size, even a temperature fluctuation of 0.05 of a degree causes a readable difference in volume. When this temperature control can be obtained, the readings are made very simple, for the rise in the manometer of the test over that of the control measures directly the amount of oxygen consumed by the seeds. The CO_2 may be determined by titration of the NaOH, or by the use of a third desiccator in which seeds but no CO_2-absorbent are placed.

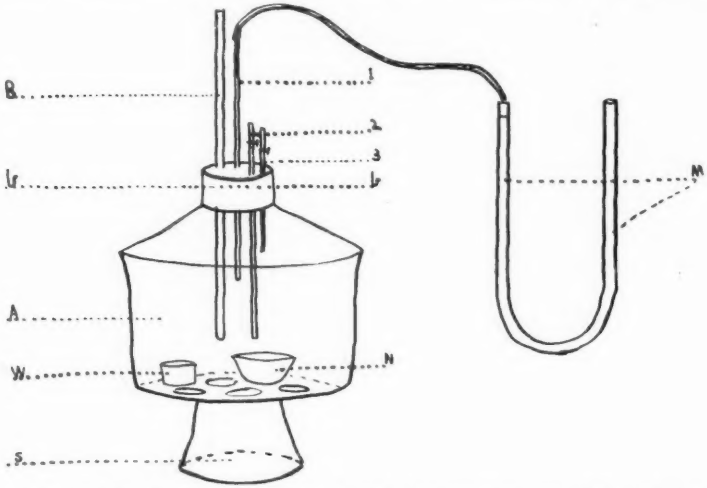

FIG. 3.—Respiration apparatus: *A*, large desiccator with stoppered lid, seeds to be tested placed at *S*; *W*, small container for water to keep air saturated; *N*, dish with wide surface for NaOH; rubber stopper at top is fitted with Beckman thermometer *B* and three small glass tubes 1, 2, and 3; tube 1 connects interior of desiccator with loose arm manometer *M*, tube 3 is exit for escape of air when NaOH is added at tube 2, which with stopcock serves for introduction of NaOH to dish *N* below; desiccator immersed in constant temperature water bath to line *b*, and NaOH added from burette after desiccator is closed and in place, to protect from CO_2 of air as much as possible.

If this is under the same temperature control as the other two, the O_2 and CO_2 difference can be read directly from this manometer. Then, knowing the value of the O_2, the CO_2 may easily be determined, and the ratio CO_2/O_2 obtained if desired. For the purposes of this experiment, however, the respiration ratio was not of interest, and therefore only two chambers were used, one for a control and one for the determination of the rate of oxygen absorption. These were immersed in a Freas water bath set at 25° C.

The fluctuation in temperature between the two, as shown by the Beckman thermometers, was negligible. Twenty-five cc. of 4N *NaOH* was used in both vessels, and about 30 cc. of water was kept in the water dish at all times. The amount of oxygen consumed by the dry seeds was found to be too small to be read hourly on the

TABLE XII

RESPIRATION OF GERMINATING WHEAT SEEDS, 65 GM. OF DRY SEEDS USED

Hours	Condition	Reading of CO_2 (cc.)	Hours	Condition	Reading of CO_2 (cc.)
48	Dry	2.0	8	Germination	4.65
1	Germination	1.1	9	Germination	5.3
2	Germination	1.0	10	Germination	5.4
3	Germination	2.1	11	Germination	7.0
4	Germination	3.2	12	Germination	7.0
5	Germination	3.6	13	Germination	10.1
6	Germination	4.1	14	Germination	17.8
7	Germination	4.0			

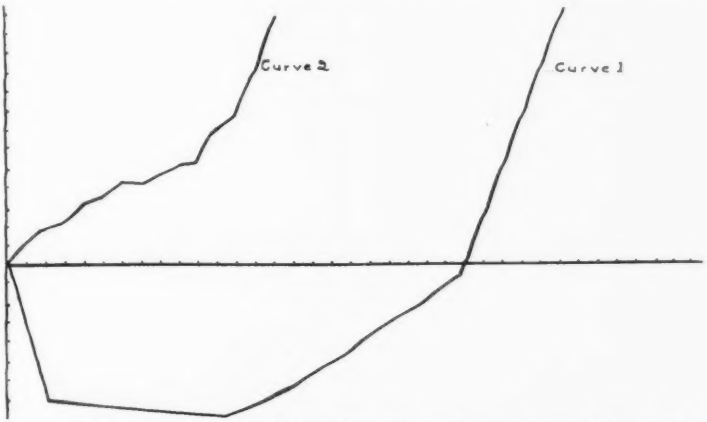

FIG. 4.—Comparison of typical catalase and germination curves during first 15 hours of germination of wheat at 23°–25° C.: curve 1, catalase, plotted from data in table IV *B*; curve 2, respiration, plotted from data in table XII.

manometer. The seeds were therefore left in the chamber with the absorbent for 48 hours, and the *NaOH* then titrated with N/10 HCl to determine the amount of CO_2 absorbed. The value from the control vessel was subtracted from the test, and the result calculated to cc. of CO_2 *equivalent, at standard temperature* and pressure. Table XII shows the results.

These data indicate strikingly that respiration increases tremendously from the first hour of germination. At no time is there any tendency for the respiration rate to decrease, or fall below that of the dry seeds. This shows that in early germination, catalase and respiration are not only not parallel, but are for a time running counter to each other, one decreasing while the other increases.

CATALASE IN DEVELOPING OVULES.—Since this unsuspected drop in catalase occurs in germinating seeds, it was thought worth while

TABLE XIII

RESPIRATION OF GERMINATING CLOVER SEEDS, 50 GM. PER TEST

Hours	Condition	Reading of CO_2 (cc.)
48	Dry	1.2
1	Germination	2.1
2	Germination	9.5
3	Germination	16.5
4	Germination	Too large to measure

TABLE XIV

GOLDEN WAX BEAN

No.	Condition	Wet weight per ovule	Dry weight per ovule	O_2 per gm. wet weight	O_2 per gm. dry weight	O_2 per ovule
1	Early green	0.0115	0.00167	150.9	8987.5	1.74
2	Early yellow	0.082	0.0095	113.0	7919.8	75.4
3	Late yellow	0.826	0.4910	80.6	328.5	161.2
4	Ripe, dry pod	0.82	0.6620	57.8	175.5	47.8

to survey the general trend of the curve of catalase activity in ripening seeds as well. Determinations were made on rye and wheat seeds throughout the period beginning just before anthesis and up to maturity, and also on wax beans from the earliest stage after blossoming that the ovules were large enough to be handled, until the pod was ripe and dry. In these tests the weight and number of seeds used in an individual test had to be shifted considerably from time to time, to bring the O_2 delivery within a range of 15–30 cc. Results were corrected and computed per gram of wet weight, dry weight, and per ovule.

Catalase in developing seeds is here shown to decrease abruptly per dry weight as the ovule develops, and to decrease also per wet weight, but less abruptly during early stages, with a more marked drop during the last few days when the moisture content is decreasing so rapidly. The data per ovule show an increase up to a central point, after which there is a rapid decrease.

TABLE XV

SPRING WHEAT

No.	Condition	Age in days	Wet weight per ovule	Dry weight per ovule	O_2 per gm. wet weight	O_2 per gm. dry weight	O_2 per ovule
1.....	Before anthesis	− 2	0.0016	0.00028	161.3	8965.0	0.258
2.....	Anthesis	1	0.0042	0.0010	162.3	6763.0	0.650
3.....	After anthesis	4	0.0148	0.0043	217.0	958.4	3.28
4.....	Early green	8	0.0238	0.0055	197.3	858.0	4.03
5.....	Later green	13	0.0300	0.0110	212.0	649.2	8.05
6.....	Slightly yellow	15	0.0400	0.0220	41.0	131.0	5.30
7.....	Yellow	23	0.0350	0.0210	71.0	117.8	2.50

TABLE XVI

RYE

No.	Condition	Age in days	Wet weight per ovule	Dry weight per ovule	O_2 per gm. wet weight	O_2 per gm. dry weight	O_2 per ovule
1.....	Before anthesis	− 3	0.0015	0.00022	200.9	1339.3	0.3
2.....	Anthesis	1	0.0028	0.00046	154.76	930.6	0.44
3.....	After anthesis	6	0.0109	0.00228	186.5	888.0	2.03
4.....	Early green	10	0.0274	0.00359	229.0	907.3	6.00
5.....	Later green	24	0.0400	0.00820	227.2	1108.3	9.09
6.....	Full grown	30	0.0493	0.00278	118.5	209.9	5.85
7.....	Slightly yellow	37	0.0450	0.00223	82.73	166.1	3.14
8.....	Yellow, dry	43	0.0300	0.02500	92.2	121.2	2.65

Discussion

There have been as yet no actual data to prove that catalase does have a function and is not a mere useless by-product of cell activity. LOEW's (29) idea of the protective function of catalase had nothing but a certain amount of probability to support it. In the data here presented, however, there seem to be indications that catalase must have an actual function in the cell. It is certainly used up in cell activity. When growth processes increase suddenly, the reserve catalase from the dry seed is acted upon and greatly

decreased before the mechanism that produces the enzyme is able to get into a comparable rate of activity. If hydrogen peroxide be formed in the cell and acted upon by catalase, as LOEW suggested, it is conceivable that in the sudden increase of respiration with germination, a rapid production of hydrogen peroxide might result, and the reserve catalase be quickly decreased, just as was observed.

It is shown by these results that catalase cannot be considered as part of the respiration mechanism, since one can decrease while the other increases. If catalase were directly produced as a by-product in the process of respiration, the two curves would have to parallel each other closely, and could not show the radical departure shown in these experiments. If it were instrumental in initiating respiration, or were part of it, it would not decrease in the absence of oxygen, as it is shown to do in table X, during prolonged soaking when aerobic respiration is almost entirely cut off, nor could the respiration curve continue to rise while the catalase curve falls.

The fact that it does decrease to practical exhaustion during prolonged soaking in oxygen-free water, however, shows that catalase must be at least indirectly related to the presence of oxygen and oxidation. It is rapidly destroyed in the early growth changes that occur, both in the germinating and the soaking seeds. In the latter case, however, under anaerobic conditions the normal course of the germination process is soon retarded. The seeds do not fully germinate, although a few succeed in breaking the testa slightly. The germination process is therefore completely inhibited by the oxygen starvation. This inhibitory effect might explain the fact that the decrease of catalase at first is slightly less rapid in the soaked seeds than in the germinating ones (tables IV *A*, and X *A*). In the case of the soaked seeds the basic life processes, comparable with those that go on so very slowly in the dry seed, continue throughout the following weeks, during which the seed remains viable, but is living by anaerobic respiration only. During this period the catalase continues to be used up (table X), and when this reserve is practically exhausted the germination tests show that viability also is practically gone. When oxygen is present for aerobic respiration, as in germination, a new supply of catalase is soon produced and the curve rises again, but if it is absent no

new supply forms and the reserve is in time depleted. From this it would seem that the amount of catalase depends distinctly, although indirectly, upon aerobic respiration.

From the experimental work here presented, the most plausible theory of catalase formation in the plant seems to be that it is formed as an enzyme according to the theory of need, the presence of the substance which it attacks acting as a stimulus for the production of the enzyme. In this case the substance it attacks would be, according to LOEW's hypothesis, hydrogen peroxide, or if not that, some other substance as yet undiscovered, which can react with catalase, and which is produced in the process of aerobic respiration.

Under this hypothesis the decrease of catalase with germination could be explained by supposing the catalase reserve to be used up, in attacking the respiration products, faster than it is produced. The high stimulation of this suddenly increased quantity of hydrogen peroxide (or other similarly behaving substance) would then lead in a short time to the increased production of catalase observed, just as in the ripening seed the falling off of the rate of metabolism would decrease the stimulation of the catalase producing mechanism, with a resulting decrease in catalase (tables XIV, XV, and XVI). The almost complete exhaustion of catalase in the soaked seeds then would be attributed to the using up of the reserve under anaerobic conditions when oxidation is slowly decreasing. The catalase stimulating substance decreases with decrease in oxidation, and therefore catalase falls off also. It would also decrease with decrease of rate of the metabolism which produces it.

After the consideration of these facts it is clear that the use of catalase as a direct measure of metabolic rate is valid only within certain limits. If catalase is formed as a response to some product of respiration, its curve will always follow, never precede, the respiration curve; that is, during comparatively slow changes in respiration rate, catalase will rise more slowly and fall more slowly than respiration, while in sudden changes it may actually decrease (or increase, doubtless) when respiration is doing the opposite. Catalase, therefore, could not be used as an indicator of respiration or metabolic rate immediately following a change in conditions that affects respiration suddenly. During slow changes

in respiration rate, however, it could at least afford a rough measure of oxidation rate, and when conditions are stable for respiration it could be used as a very convenient and reasonably accurate measure of this process.

Summary

1. Catalase decreases in early germination stages in six kinds of seeds tested. The initial decrease is followed by the rise generally reported for germination.

2. Catalase decreases slowly, almost to exhaustion in prolonged soaking of seeds in oxygen-free water.

3. There is a decrease of catalase in both the embryo and the endosperm of germinating wheat. The subsequent rise occurs not only in the embryo but also in the endosperm, although in a much less marked degree.

4. A simple respirometer is described, in which large quantities of material can be used, and consecutive readings taken at will on the same sample. By direct reading of the manometers both the oxygen and carbon dioxide can be determined without titration and with practically no calculation.

5. There is a large and immediate rise in respiration when seeds are put in germinating conditions. The early curve of respiration, therefore, diverges widely from the typical germination curve of catalase.

6. Catalase in ripening seeds decreases per unit both of wet and of dry weight. It increases per ovule as the seed grows, but after the ripening process begins, it decreases rapidly with decrease of water content.

7. The production of catalase is indirectly connected with oxidation, and LOEW's theory of the function of catalase is advanced to explain the facts, if the presence of some substance such as hydrogen peroxide, on which catalase acts, be formed to act as a stimulus for the production of the enzyme.

8. Catalase could be used as an indicator of metabolism only in cases where there is no rapid change in respiration.

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LITERATURE CITED

1. AMBERG, S., and WINTERNITZ, M. C., The catalase of sea urchin eggs before and after fertilization, with special reference to the relation of catalase to oxidation in general. Jour. Biol. Chem. **10**:295–302. 1911–12.
2. APPLEMAN, CHAS. O., Some observations on catalase. BOT. GAZ. **50**:182–191. 1910.
3. ———, Relation of oxidases and catalase to respiration in plants. Amer. Jour. Bot. **3**:223–233. 1916.
4. ———, Respiration and catalase in sweet corn. Amer. Jour. Bot. **5**:207–209. 1918.
5. BACH, A., and CHODAT, R., Über den gegenwartigen Stand der Lehre von den pflanzlichen Oxydationsfermente. Biochem. Centralbl. **1**:417–421. 1901.
6. BALY, E., HEILBRON, I., and HUDSON, D., The photosynthesis of nitrogen compounds from the nitrates and carbon dioxide. Jour. Chem. Soc. London **121**:1078–1088. 1922.
7. BATELLI and STERN, quoted from KASTLE (**27**).
8. BECHT, FRANK C., Observations on the catalytic power of blood and solid tissue. Amer. Jour. Physiol. **48**:171–191. 1919.
9. BECKING, L. G. M. B., and HAMPTON, H. C., Measurement of the catalytic power of catalase. Amer. Jour. Bot. **7**:261–274. 1920.
10. BURGE, W. E., Comparison of catalase content of breast muscle of wild pigeons and of bantam chicks. Science **46**:440. 1917.
11. ———, Why chloroform is a more powerful and dangerous anesthetic than ether. Science **46**:618. 1917.
12. ———, Effect of acids, alkalis, and salts on blood catalase. Amer. Jour. Physiol. **52**:364–376. 1920.
13. ———, Changes in catalase content during the life cycle. Amer. Jour. Physiol. **56**:29–32. 1921.
14. BURGE, W. E., and LEICHSENRING, J. M., Effect of cold on catalase content of blood. Amer. Jour. Physiol. **56**:408–414. 1921.
15. CHOATE, HELEN, Chemical changes in wheat during germination. BOT. GAZ. **71**:409–425. 1921.
16. CROCKER, WM., and HARRINGTON, G. T., Catalase and oxidase content of seeds in relation to their dormancy, age, vitality, and respiration. Jour. Agric. Res. **15**:137–174. 1918.

17. DAKIN, H. D., Physiological oxidations. Physiol. Rev. **1**:394–420. 1921.
18. DELEANO, N. T., Recherches chimiques sur la germination. Centralbl. Bakt. und Par. **24**:130–146. 1909.
19. DUTCHER, A., Vitamine studies. Catalase activity in avian polyneuritis. Rev. in BOT. GAZ. **67**:170. 1919.
20. ENGLER, C., and HERZOG, R. O., Zur chemischen Erkenntnis biologischer Oxydationsreactionen. Zeit. Physiol. Chem. **59**:327–375. 1909.
21. EWALD, W., Die Physiologie der oxydativen Blutfermente. Archiv. Gesammt. Physiol. **116**:334–346. 1907.
22. HEINICKE, A. J., Factors influencing catalase activity in apple leaf tissue. Cornell Agric. Exp. Sta. Mem. **62**:5–19. 1923.
23. HERTZLIKA, quoted from KASTLE (**27**).
24. ISSAJEW, W., Über die Hefekatalase. Zeits. Physiol. Chem. **42**:102. 1906.
25. JOLLES A., and OPPENHEIM, M., Beitrage zur Kenntnis der Blutfermente. Virchow's Archiv. **180**:185–225. 1905.
26. JONES, H. A., Physiological studies of the maple seed. BOT. GAZ. **69**:127–152. 1920.
27. KASTLE, J. H., The oxidases. Public Health and Marine Hospital Serv. U.S. Hygiene Lab. Bull. **59**:139–140. 1909.
28. LESSER, E. J., Zur Kenntnis der Katalase. Zeit. Biol. **49**:575–583. 1907.
29. LOEW, O., A new enzyme of general occurrence. Dept. Agric. Rep. **68**: pp. 47. 1901.
30. ———, Zur Theorie der Katalasefunktion. Archiv. Gesammt. Physiol. **128**:560–564. 1909.
31. MOORE, W., and WILLAMAN, J. J., Studies on greenhouse fumigation with hydrocyanic acid: physiological effects on the plant. Jour. Agric. Res. **11**:319–338. 1917.
32. MORGULIS, S., A study of the catalase reaction. Jour. Biol. Chem. **47**:341–375. 1921.
33. ———, Is catalase a measure of metabolic activity? Amer. Jour. Physiol. **57**:125–135. 1921.
34. NEUMANN-WENDER, Die Hefekatalase. Chem. Zeitung **28**:300–301; 322–323. 1904.
35. PACK, D. A., After-ripening and germination of *Juniperus* seeds. BOT. GAZ. **71**:32–60. 1920.
36. ROSE, R. C., After-ripening and germination of seeds of *Tilia*, *Sambucus*, and *Rubus*. BOT. GAZ. **67**:281–308. 1919.
37. ROSENBERG, ROSA, Über die Rolle der Katalase in den Pflanzen. Ber. Deutsch. Bot. Gesell. **28**:280–288. 1910.
38. SCHAFFER, P., Some observations on the enzyme catalase. Amer. Jour. Physiol. **14**:299–312. 1905.
39. SEYMOUR, R. J., The relation of catalase to heart activity. Amer. Jour. Physiol. Balt. **51**:525–529. 1920–21.

40. SENTER, Das Wasserstoffsuperoxydzersetzende Enzym des Blutes. Zeitschr. Physikal. Chem. **44**:257–263. 1903; **55**:673–705. 1905.
41. SHERMAN, HOPE, Respiration in dormant seeds. BOT. GAZ. **72**:1–30. 1921.
42. SHULL, C. A., and DAVIS, W. B., Delayed germination and catalase activity in *Xanthium*. BOT. GAZ. **75**:268–281. 1923.
43. SPITZER, W., Die Bedeutung gewisser Nucleoproteide für die oxydative Leistung der Zelle. Pfluger's Archiv. **67**:615–656. 1897.
44. STEHLE, R. L., and MCCARTY, A. C., The alleged relation of catalase to animal oxidations. Jour. Biol. Chem. **42**:269–272. 1920.
45. USHER, F. L., and PRIESTLY, J. H., Mechanism of carbon assimilation. Proc. Roy. Soc. Lond. **77**:369–376. 1905; **78**:318–327. 1905.
46. ZALESKI, W., and ROSENBERG, A., Zur Kenntnis der Rolle der Katalase in den Pflanzen. Bot. Centralbl. **119**:431. 1912.
47. ZEIGER, R., Zur Kenntnis der Katalase der niederen Tiere. Biochem. Zeit. **69**:39–110. 1915.

FLOWERS AND INSECTS. XXIII

CHARLES ROBERTSON

The flower groups are divided into (1) a social set, whose flowers are in heads, spikes, or close umbels, so that insects pass from one to another without taking wing or climbing; and (2) a non-social set. The latter contains 54.4 per cent of the flowers, and is divided into Ma, 121 long-tongued bee flowers, and 11 adapted to birds, hawk-moths, and butterflies; and Mi, 100 short-tongued bee flowers, and 6 adapted to flies. The social set includes Mas, 82 long-tongued bee flowers; Mis, 94 short-tongued bee flowers; and Pol, 23 visited by miscellaneous short-tongued insects.

The insect visits (13,971 pollinating and 850 non-pollinating) were first distributed under MÜLLER'S (8) classes. These were then divided into non-social and social sets, and the visits were distributed again under them. Of 9 cases, maxima under Hb fell under non-social Hb in 6. In 27 other cases, however, maxima under B, AB, or A always fell under the social sets. As a criterion the social or non-social condition is of primary importance, while that of nectar concealment is secondary.

The flowers were also divided into a group of 235 plants observed before July, and one of 262 plants observed after June. Visits to these were first distributed under MÜLLER'S classes. Before July there are maxima under B, AB (including Po), and A. After June there is a slight increase in Hb, including O and F, and a marked increase in B′. The visits were also distributed under the new classes. The early group shows maxima of the simplest flowers, Mi and white colors. These, along with Mis, decrease in the late group, which shows maxima of Ma and red (all dark colors), and a great increase in Mas. The specialization of the late group is marked by the increase of the social flowers from 38.7 to 54.1 per cent.

When B′ as an element of the flora changes from 8.0 to 28.2 per cent, in 94.2 per cent of the cases the maxima of the insect groups change from the four other dominant classes to B′. The

maxima of the same insects, when distributed into early and late sets of the new categories, in 62.8 per cent of the cases fall under the same classes.

Importance of bees and method of investigation in anthecology

Of 2288 visitors and 13,971 visits, the Lepidoptera show 7.3 and 8.5 per cent, the flies 31.2 and 27.0, and the lower Hymenoptera 26.0 and 16.8. The bees, however, form 22.9 per cent of the species and make 43.3 per cent of the visits. In 22 cases of dominant orders, families, and genera, bees show a gain of visits over species, except in *Eupatorium*. Flies show a little gain for visits in only three cases, the lower Hymenoptera in six, and the Lepidoptera in four.

SPECIES AND INDIVIDUALS.—After a summary of insect visits to different sets of Compositae, MÜLLER (8) states:

> This table proves clearly that in Compositae as the honey becomes more deeply placed the visits of more highly specialized bees increase, while, in spite of the exposed situation of the pollen, the visits of flies fall off. This is true of the number of species of visitors; but if it were possible to record the number of visits paid by each species, the preponderating influence of bees would be still more apparent.

This statement involves a criticism of basing conclusions on lists of species, a method which is quite unsatisfactory because it results in an underestimate of the importance of bees.

It is obvious that, if flowers are modified by their visitors, the number of individuals of each kind and the probability of repeated and diligent visits are very important. In the quotation MÜLLER implies that it is impossible to record the number of visits paid by each species. KNUTH says (5): "It is almost impossible to count all the individual visits that a conspicuous flower receives during a considerable period of observation, when the weather is fine." If one is making a list of the species which visit a given flower, one will look out for species not noted before, the rare and exceptional ones, and constantly exaggerate their importance. The longer the list, often the more erroneous the result. Generally it is easy to add new species, but a satisfactory census can be made in

one season. In the case of *Aster ericoides villosus*, the present list shows flies as 39.7 per cent of the visitors, and bees 30.8. In a census list the flies were 41.9 per cent of 124 species, but the bees 33. Of 1126 individuals, however, the flies were 33.5 per cent and the bees 49.2. When bees are 22.9 per cent of the total species and flies 31.2, in the case of flowers adapted to miscellaneous short-tongued insects, it is probable that, with continued watching, the percentage of fly species will increase. This may account for the fact that in the 1896 list of 116 species (11) the flies were 36.2 per cent, while in the present list of 292 species flies are 39.7.

In the case of *Rudbeckia subtomentosa*, the long-tongued bees showed 14.4 and the short-tongued 17.1 per cent of 76 species. Of 669 individuals, the former were 6.5 per cent and the latter 40.5. In the case of 87 species, taken on *Symphoricarpos orbiculatus*, bees were 35.6 per cent and wasps 34.4; while of 1065 individuals, bees were 76.3 per cent and wasps 9.8. Of 45 species, taken on *Lippia lanceolata*, bees were 33.3 per cent, flies 42.2, and wasps 8.8; while of 288 individuals, bees were 44.7, flies 29.1, and wasps 3.4 per cent. In 60 cases in which insects were taken as they came, bees gained in percentage of individuals over that of species in 53. Of 405 species observed on 41 plants, bees were 31.6 per cent; but of 7391 individuals, bees were 57.4 per cent. Insects of the other groups declined.

Forty-three plants with *Diptera maxima* showed for species and visits of bees 21.1 and 25.5; of flies 36.3 and 42.9. Twelve of these showed for visits and individuals of bees 32.9 and 49.5; of flies 42.9 and 35.3, a gain for bees and a loss for flies. Twenty-six plants with lower Hymenoptera maxima showed for species and visits of bees 20.2 and 21.4; of lower Hymenoptera 35.6 and 40.4. Seven of these showed for visits and individuals of bees 28.3 and 65.7; of lower Hymenoptera 37.2 and 14.6, a gain for bees and loss for lower Hymenoptera.

THE COMMONEST VISITOR.—In lists of species some are marked "abundant" or "frequent." What these terms amount to is shown when the individuals are counted. A bee, which makes only one in a list of species, often shows a remarkable importance as a pollinator. Sometimes this may be owing to the fact that

the flower blooms when the bee is most active. At Inverness, Florida, 1000 individuals taken on *Laurocerasus caroliniana* showed 527 of *Opandrena scutellaris*. Another 1000 on *Fagara clava-herculis* showed 256 individuals of *Colletes thoracicus*, and only three of another *Colletes*. *Odontalictus capitosus* formed 65.3 per cent of 300 individuals taken on *Berlandiera subacaulis*, and 17.8 per cent of 599 individuals on *Thysanella fimbriata*. The first 100 individuals on *Claytonia virginica* showed 39 specimens of the oligolectic *Ptilandrena erigeniae*. In 85.5 per cent of local flowers, on which the individuals were taken, the commonest visitor was a bee which showed 3.5 per cent of the visits and 20.7 per cent of the individuals.

ORDINARY AND CENSUS VISITS.—When the visitors are captured without discrimination instead of being observed to ascertain the number of species, bees usually show higher percentages of species and visits. The visits to 29 plants showed 32.1 per cent of bees, but the same plants, when the individuals were taken as they came, showed 38.8 per cent of bees.

To make a correct statement regarding a flower visited by miscellaneous insects, a census of species and individuals taken without discrimination ought to be made about every other day throughout the blooming season.

APOCYNACEAE AND ASCLEPIADACEAE.—In 11 species bees showed 21.6 per cent of 273 visits without pollinia, and 32.1 per cent of 308 visits with pollinia.

DICLINOUS FLOWERS.—In 8 species of diclinous plants bees made 37.8 per cent of 420 visits to staminate or pistillate flowers only, and 63.4 per cent of 194 visits to both kinds.

Imperfect adaptation of flowers to insects

Flowers may be regarded as showing imperfect adaptation whenever they fail to exclude visitors which are unable to reach the nectar in the normal way, or which obtain the nectar or pollen without effecting pollination. More than 38.2 per cent of the flowers considered here show such imperfect adaptation. When the indigenous flora and the insects which depend upon the flowers are being rapidly exterminated, the relations are inevitably more

or less out of joint, and cases of imperfect adjustment become more frequent. As regards the existence of flowers which have no unbidden guests, however, or of floral structures which always effectually exclude such guests, it is hardly an exaggeration to state that there are none.

Flowers with exposed nectar admit the smallest insects. Such flowers are usually small, but some of their visitors are so minute that they can obtain the nectar without pollination. The larger the flowers, the greater the possible number of unbidden guests. On *Melanthium virginicum* fifteen visitors were observed sucking without carrying pollen. As many as twenty Chironomidae were observed on a single nectary. *Liparis liliifolia* and *Caulophyllum thalictroides* have similar useless visitors.

Many flowers whose nectar is partly concealed or rather deep seated admit insects too small to touch the anthers and stigmas, such as *Polygonatum commutatum* and *Geranium maculatum*. In some of these there appears to be a well marked lack of correlation between the size of the flower and the depth of the nectar.

In *Iris* (15) butterflies insert their proboscides between the sepals and petaloid styles and steal the nectar. In *Lobelia* (14) the stamen tube is not completely closed at the base. In *L. cardinalis*, *Bombus americanorum*, which cannot reach the nectar in the legitimate way, obtains it by inserting its proboscis through the slits.

In the case of *Cypripedium pubescens* two species of Ceratinidae were seen entering the labellum and making their exit in the normal way without extracting the pollen. These bees were evidently too small.

The bearded violets are adapted to small bees which reverse and insert their proboscides under the anthers and stigma. Some bumblebees, long-tongued flies, and Lepidoptera fail to reverse and so extract the nectar without effecting pollination.

Flowers with concealed pollen may be pollinated by Lepidoptera when the anthers are inclosed in slender tubes, as in *Phlox*, but in other cases they usually fail to exclude such insects, although they are not pollinated by them.

Personate, papilionaceous, closed flowers, like *Gentiana Andrewsii*, *Collinsia verna*, and *Dicentra cucullaria*, and flowers with the

throat closed by tufts of hair, like *Seymeria macrophylla*, or with the stamens protected by petals, as in *Delphinium tricorne*, usually exclude all unbidden guests, except Lepidoptera and siphonate Diptera. *Linaria vulgaris* is adapted to bumblebees, which are heavy enough to pull down the palate easily, less so to smaller bees, which have to squeeze in sideways, and not at all to butterflies, which steal the nectar.

Apocynum fastens its pollinia to the maxillary and labial palps of bees and wasps, but can neither utilize nor exclude flies and Lepidoptera.

Asclepias admits many insects which get the nectar without extracting the pollinia, and others which have difficulty in breaking the retinacula or freeing their claws from the slits, and so often are entrapped and killed. Eleven species of Apocynaceae and Asclepiadaceae fail to utilize 46.9 per cent of the visits made to them.

As a rule visitors of one form of diclinous flowers are admitted here as legitimate on the assumption that they probably occur on both forms, but in elucidating floral mechanisms, only those visitors which were found on both forms should be admitted. The Syrphidae and Coleoptera show a marked preference for the staminate flowers. In 614 visits to eight species of diclinous flowers, 68.4 per cent were to one form.

In the case of the staminate flowers of *Diospyros virginiana*, the Syrphidae made 18.7 per cent of the visits, avoiding the pistillate. Short-tongued bees made 62.7 per cent of the visits to staminate flowers, and 33.3 of the visits to both forms, but the two forms look as if they belonged to distinct species. The staminate are smaller and have less deep seated nectar.

The staminate flowers of *Salix humilis* have been observed to attract hive-bees in such numbers as fairly to monopolize them, while the pistillate flowers were visited by an entirely different set of insects.

Dichogamous flowers which are discharging pollen are often more attractive to insects than those in the other stage. *Impatiens biflora* in the first stage induces pollen visits of some long-tongued bees which avoid the flowers with receptive stigmas. *Campanula americana* attracts *Oligotropus campanulae* before the stigma opens, but not after. *Lobelia siphilitica* invites the visits of little bees,

which collect the pollen which is pushed out of the anther tube before the stigma appears. *Monarda bradburiana* admits small bees to which the nectar is inaccessible, but they collect pollen directly from the anthers, avoiding the old flowers. Thirty-five species of flowers have been observed to attract bees which collect pollen in the first stage but avoid them in the second.

Personate, papilionaceous, and other closed flowers usually exclude pollen-hunting insects which cannot reach the nectar, but *Dicentra cucullaria*, *Delphinium tricorne*, and *Trifolium pratense* sometimes fail to do so in spite of hindering structures.

As a rule flowers with exposed pollen and deep seated nectar, like *Abutilon Theophrasti* and *Mertensia virginica*, are visited for pollen by small insects, usually Halictidae and Syrphidae. On the bird flower, *Tecoma radicans*, and the hawk-moth flower, *Datura tatula*, bees were observed collecting pollen.

Large pollen flowers, such as *Tradescantia*, *Rosa*, and *Cassia*, which are adapted to large bees, are commonly visited by small bees, Syrphidae, and beetles which do not often effect pollination.

Ordinary flowers with exposed pollen probably attract more Syrphidae than any other useless visitors. These flies are light and rest so superficially on the flowers that they can eat the pollen without carrying it.

Many flowers fail to exclude beetles, which eat the pollen or gnaw and often completely destroy various flower parts.

Some flowers are visited by *Megachile brevis*, when she cuts circular pieces out of the petals to line her nests.

In the Alps, flowers whose nectar it could not obtain in the normal way are often perforated by *Bombus mastrucatus* (MÜLLER 9). In the Tyrol, SCHULZ (12) found 125 species of flowers perforated by *Bombus terrester*. This may be a result of the competition with the domestic hive-bee on flowers to which this bumblebee would normally confine its visits. In observing the visits of bumblebees to 278 insect flowers, none was ever seen perforating, and none obtaining nectar in an altogether irregular way except on *Lobelia cardinalis*. No flower was seen to be perforated except by *Leionotus foraminatus* and *L. dorsalis*.

Flower buds often contain nectar before they open. I have seen the rubythroat thrust its bill into buds of *Lonicera sempervirens*,

and *Bombus consimilis* force its proboscis into buds of *Triosteum perfoliatum*. This may help to account for cases like *Gentiana Andrewsii*, whose flowers never open.

Homogamous flowers, which are so small that insects cannot obtain the nectar or collect the pollen without effecting pollination, seem to be best adapted to utilize all of the visitors which they attract.

The useless visits to flowers were made by Lepidoptera, Halictidae, Syrphidae, and Coleoptera, respectively 20.5, 19.3, 17.0, and 14.4 per cent of the total, together 71.2 per cent. These useless visits, however, are only 5.7 per cent of the total visits observed. Of these visits, 41.1 per cent are to Ma, 31.3 to Mas, and 46.3 to red, that is, to the flowers with the deepest seated nectar.

Characteristics of insect flowers

NECTAR.—The existence of "nectar covers" is not obvious. There are structures which have that effect, but they exist also in pendulous flowers where they would not be needed. Nectar, together with pollen and other parts of the flower, is protected by the closing of the flower in rainy weather. The structures called "nectar covers" should probably be regarded as serving to exclude the less efficient pollinators.

Some flowers, as *Orchis*, are said to have no free nectar in their spurs. Insects are supposed to obtain the nectar by boring into the juicy cellular tissue. In *Habenaria leucophaea* and *Orchis spectabilis*, nectar rises so that the height can be told from the outside. This hardly supports the idea that the nectar is in the cellular tissue.

That glandular hairs in pollen flowers (KERNER 4) supply means of moistening the pollen is altogether doubtful. Such hairs exist in *Steironema*, which *Macropis steironematis* visits for pollen, but this bee can easily moisten the pollen with honey obtained from the several flowers which it visits for nectar.

POLLEN.—Pollen often influences the visits of insects to flowers. Female bees, with their plumose, hairy coats covered with it, are the most certain of all insects in pollinating, so effective that many flowers have lost their nectaries and usually are visited exclusively by female bees. After the bees, the Syrphidae and beetles are the

most frequent visitors for pollen, which they can eat without much certainty of effecting pollination.

KERNER (4) has given seventeen arrangements for protecting pollen. Pollen is certainly sheltered by a pendulous position of the flower, but it does not follow that any plants were selected because their pollen was protected by that position. The closing and nodding of flowers at night and in bad weather may be regarded as having some connection with natural selection to protect the floral organs, but not the pollen exclusively. The spathe of *Arisaema triphyllum*, mentioned by KERNER as protecting pollen, also protects the flowers which have pistils, but no pollen. "In *Podophyllum peltatum* the pollen is sheltered by the bell-shaped flower, but in addition to this, the peltate foliage leaves are also spread out over the flowers and act as umbrellas."

The anther closing, or remaining closed in moist weather, and arrangements in which the pollen is retained until dislodged by the legitimate visitors, may be regarded as pollen protective: the triggered anthers of *Gerardia grandiflora*, the milked anthers of *Cassia*, the bellows-like anthers of *Rhexia*, the anther cones of *Viola* which shed when the style is touched, the keel of Papilionaceae, galea of Labiatae, closed flowers, etc. Pollinia are usually retained until dragged out by insects, but the protective advantage is rather incidental.

In wind flowers the pollen is light and dry, and easily carried by the wind. In some insect flowers the pollen is shed upon the visitors and is dry and powdery; commonly it is rough and more or less viscid. Sometimes the grains are connected by threads, as in *Oenothera*, or the contents of an anther cell may be united into a pollinium.

In *Cassia chamaecrista* some anthers serve to supply pollen to the guests, and others to furnish it for cross pollination.

Ordinary flowers dust the insect with pollen quite indefinitely, but mainly on the lower side. Some gonous zygomorphous flowers dust the insect on the back, some on the ventral surface, and some on the side. In Florida an *Astragalus* was observed whose keel was always turned to one side, so that it dusted the bees only on the under side of the left cheek. *Orchis spectabilis* fastens its pollinia

to the bare clypeus of female bumblebees, *O. pyramidalis* (1) to the upper side of the proboscides of Lepidoptera, *Habenaria leucophaea* to the tongues, and *H. orbiculata* (3) to the eyes of Sphingidae, *Spiranthes gracilis* to the maxillary galea of bees, *Calopogon parviflorus* to the first dorsal segment of the abdomen. *Asclepias Sullivantii* fastens its pollinia to the claws, *A. verticillata* to the tarsal hairs, *Acerates floridana* to the ventral hairs, *Gonolobus laevis* to the tongues, and *Apocynum* to the palpi.

In addition to nectar flowers and pollen flowers may be distinguished the fruit flowers of *Yucca* and the gall flowers of *Ficus*. In these the fruit is partly injured by the pollinator.

COLOR.—Usually the petals, sometimes the sepals, or both, serve as advertising structures, and are variously colored.

Nectar guides, colored marks contrasting with the ground color of the flower, and indicating the situation of the nectar, were designated by SPRENGEL (13). They facilitate effective insect visits. Usually flowers with these guiding marks are zygomorphous, or otherwise complicated, or with deep-seated nectar. They are common in Papilionaceae, Lamiales, and Personales. On *Lantana odorata*, butterflies insert their proboscides into the flowers with guides, but avoid the old flowers, in which the guides are obliterated. It seems evident that the insect can distinguish these flowers only by the color.

Pollen flowers also have guiding marks called path finders. They seem to occur only in zygomorphous flowers, *Desmodium*, *Cassia*, and *Calopogon*. In *Desmodium* bees insert their heads under the specially marked vexillum when they force down the wings. In *Cassia* the path finders mark the location of the short stamens. Colored marks are present in *C. chamaecrista*. These are absent in *C. marilandica*, but three stamens are reduced to broad, dark scales to take their places.

Evidently the importance of the colors of flowers has been exaggerated. They should be regarded as advertisements rather than attractions. Insects are guided by the colors in their search for floral food, but they do not visit flowers to look at their colors. To humanize insects, it may be stated that they visit flowers for the following purposes: (1) for nectar; (2) in search of nectar which

the flowers do not contain (*Lycaena*, *Coelioxys*, *Anthrax* on *Desmodium*); visits to sham-nectar flowers; visits of various insects to flowers whose nectar they cannot reach; (3) for pollen, with which they provision their nests (bees); which they eat (Syrphidae, beetles, etc.); (4) for pollen which cannot be collected; female bees on sham-pollen flowers (*Calopogon*); (5) to cut holes in flowers whose nectar they cannot reach normally (*Leionotus foraminatus*); (6) to get nectar from holes made by *Leionotus;* (7) to gnaw floral organs (beetles); (8) in search of anthophilous insects (*Vespa*, *Polistes*, *Protothyreopus*, *Ochthera*); (9) for flower spiders (*Sceliphron cementarius*); (10) to lie in wait for anthophilous insects (*Phymata*); (11) in search of the females; male bees often fly around among the flowers, only approaching near enough to discover the presence of the females; (12) to deposit their eggs in the ovaries (*Pronuba*, *Blastophaga*); (13) for shelter; flies in *Arisaema;* bees in various flowers; (14) to deposit eggs so that their larvae may become attached to anthophilous hosts (*Rhipiphoridae*); (15) to cut petals to line their nests (*Megachile brevis*); (16) merely as resting places.

That insects can find nectar without any guides is shown by the great variety of species (22) that may be found on sugar trees from which the sap is exuding, on leaves covered with honey dew, or on extranuptial nectaries, 16 species on *Tecoma radicans*, 66 on *Cassia chamaecrista*, and 43 on *Strophostyles helvola*.[1]

The readiness with which insects find nectar is also shown in the case of *Monarda fistulosa*, in which nine species of bees and one wasp, which could not reach the nectar in the normal way and which consequently could hardly be regarded as attracted by the colors of the flowers, were observed sucking nectar through holes made by *Leionotus* (10).

Anemophilous flowers which have no conspicuous colors are visited by bees for pollen.

Nevertheless, that the colors of flowers guide insects to them is shown by the frequent visits of insects to flowers which do not contain anything they can get; visits of butterflies, inquiline bees,

[1] The bees taken at sugar maples were *Apis mellifera* and *Trachandrena rugosa;* those on the extranuptial nectaries were usually Halictidae. Of these insects, eleven species of lower Hymenoptera and six species of flies were never taken on any flower.

and siphonate flies to pollen flowers; of bees to sham-pollen flowers; of flies to sham nectar flowers. DELPINO (2) observed a bee working on *Anemone nemorosa* confused by the presence of *Bellis perennis*, which it often approached before recognizing its error. and I have often noticed bees confused by the presence of similarly colored flowers which were mixed among those on which they were working. On several occasions *Bombus americanorum* was observed approaching pappus heads of *Taraxacum officinale*, evidently mistaking them for flowers. LUBBOCK (6) has shown that hive-bees can distinguish colors and use the colors to enable them to return to the same place where they have found honey.

Another fact bearing on this subject is connected with the colors of spiders. The flower spiders, Thomisidae, are yellow or white, corresponding with the colors of the flowers on which they lie in wait. They have no other means of concealment, but a black jumping spider, observed on a head of *Helianthus*, formed a hiding place by tying several rays together.

A typical case showing the relations of insects to flower colors is that of the mud-dauber, *Sceliphron cementarius*. It visits for nectar 16 white, 6 yellow, and 3 red flowers, evidently aided by the conspicuous colors, but it provisions its nest with flower spiders in spite of their cryptic colors. Of the organisms from which insects get their food, flowers are almost the only ones which gain any advantage from their attentions and which facilitate their search.

COLOR PREFERENCE OF INSECTS.—The only direct observation on color preference was in the case of a hawk-moth, *Celerio lineata*, visiting a bed of petunias in which white and purple flowers were about equally abundant. It distinctly avoided the purple flowers and visited the white ones.

There are indications that insects have had color preferences which have influenced the colors of their favorite flowers, but their visits to certain colors are always complicated with other conditions. We often read about reactions of insects as if they were quite simple. To prove this simplicity we have to go back to the first generation, or show that natural selection, heredity, and experience have nothing to do with the cases.

As we pass from red through yellow to white, the visits and the average number of visits increase. The visits of long-tongued bees, of bees in general, and of Lepidoptera decline; the visits of flies, lower Hymenoptera, and short-tongued bees increase.

COLOR PREFERENCE OF BEES.—MÜLLER says: "On the whole we find red, violet, and blue colors appearing for the first time in flowers whose honey is quite concealed and which are visited by more or less long-tongued insects (bees, etc.)."

LUTZ (7) states that bees are largely given to visiting blue, purple, or violet flowers, although not totally ignoring yellow or red ones. Bees are heterotropic. They have become diversified in correlation with the diversification of the entomophilous flora. Only 42.7 per cent of indigenous entomophilous flowers bloom at the same time, and only 47.2 per cent of the bees fly simultaneously. It is not probable that 296 species of local bees are so free from competition among themselves that they do not have to trouble themselves about flowers, except those whose colors suit their fancy. Bees visit nectar-bearing flowers of all kinds, except those adapted to birds, hawk-moths, and flies, which are so constructed that they are practically excluded. Even on these, bees are apt to force their attentions, as in *Lobelia cardinalis*, *Tecoma radicans*, and *Datura tatula*. There are only three local flowers on which I am very sure bees never occur: *Habenaria leucophaea*, *Aristolochia tomentosa*, and *Trillium sessile*. If we include non-pollinating visits, the percentage of flowers visited by bees is 96.2.

In the case of the 437 local flowers the colors are red 30.2, white 39.1, yellow 30.6. The 417 flowers visited by bees are red 28.7, white 40.0, yellow 31.1. The 6063 bee visits are red 23.1, white 43.2, yellow 33.5. The flowers visited and the visits made are not very different from what might be expected without regard to color.

Of local bees only 70 (23.6 per cent) are largely given to visiting red flowers. Of their visits, 45.9 per cent are to red; of the visits of 130 other bees, 55.9 per cent are to white; while of the visits of the remaining 96 species, 52.9 per cent are to yellow.

There is a little more reason for KNUTH'S (5) statement that long-tongued bees prefer red, blue, and violet colors. In the case of 146 local species, however, only 60 (41.0 per cent) prefer red, 46.0 per cent of their visits being to that color. Forty-three show

a maximum of 50.3 under white, and 43 show a maximum of 51.4 under yellow.

The long-tongued bees show for flowers visited red 31.6, white 37.2, yellow 31.0. The visits show: red 34.0, white 34.5, yellow 31.4; so that their visits are about what might be expected without color preference.

There are not many flowers differing in colors but alike in other respects. In Ma I include 121 long-tongued bee flowers whose colors are red 56.1, white 21.4, yellow 22.3. Long-tongued bee visits (688) to these show red 52.6, white 24.1, yellow 23.2; about what might be expected without color preference. The percentages of long-tongued bee visits to the three colored sets of flowers of this class are to red 59.7, white 78.6, and yellow 73.0. Before July 29.6 per cent of the flowers of Ma are white and 20.3 yellow, after June 13.7 are white and 26.0 yellow. Visits of long-tongued bees to this class showed before July, 30.5 per cent under white and 16.0 under yellow, after June, 13.1 under white and 31.5 under yellow, so that when conditions are equal, long-tongued bees will make about the same percentage of visits to flowers of one color as to those of another. If the flowers of class Ma were separated into those with ultra violet colors and those without them, the visits to each set would correspond fairly well with the percentages of flowers. Of 30 species of bees showing maxima, or principal preferences, under Ma, 20 showed similar maxima, or preferences, under red.

In the case of the rubythroat, 82.7 per cent of the visits are to general Ma, which shows 57.5 per cent of red flowers. Of its visits, 55.1 per cent are to red, which is about what might be expected without color preference. This bird, however, seems to prefer bright red, or scarlet, all of our bird flowers being of that color.

Flowers referred to Mas are red 43.9, white 10.9, yellow 45.1. Visits of long-tongued bees (1185) show red 45.5, white 9.4, yellow 43.9. Of 100 bees showing maxima, or principal preferences, under Mas, 44 showed similar maxima, or preferences, under red, 9 under white, and 47 under yellow. Long-tongued bee visits to red, white, or yellow Mas differ less than 1 per cent.

In 87 Asterales the radiate species were classed according to the color of the rays. In general, red shows the longest tubes

and white the shortest, while yellow shows tubes of about average length. Accordingly, red shows a loss and white a greater gain than yellow. The colors are red 21.8 per cent, white 31.0, yellow 47.1; while 3939 visits of insects in general show red 13.6, white 37.4, and yellow 48.8. The visits are about what should be expected according to the depth of the tubes and without regard to the colors.

Of the visits of Bombinae, 41.1 per cent are to red flowers and 30.1 to white, while of the visits of the Halictidae 50.0 are to white flowers and 14.4 to red. There seems to be no evidence that the color is the determining condition. The inference of color preference rests on the fact that the visits of the former show a higher percentage to red flowers, and of the latter a higher percentage to white ones. If we assume that in the first place the Bombinae preferred red and the Halictidae white, the color factor must have ceased to become dominant after the flowers became modified to suit the one, and exclude, or fail to satisfy, the other. The fact that the Halictidae make 86 visits to red flowers, although they cannot obtain the nectar in the legitimate way, establishes the presumption that they would be more frequent, if they could obtain the nectar. Also, the bumblebees would visit more white flowers if these flowers afforded a more abundant supply of nectar.

Oligolectic bees might be expected to show color preferences according to the colors of their special flowers. On their visits to unrelated flowers, however, the short-tongued oligoleges of red and yellow flowers prefer white. In a similar way the nectar visits to unrelated flowers of long-tongued oligoleges show preference for red flowers without regard to the colors of the flowers which they visit exclusively for pollen. The long-tongued oligoleges in 127 pollen visits show red 23.6, white 14.1, yellow 62.2; in 142 nectar visits to unrelated flowers they show red 59.1, white 27.4, yellow 13.5. The short-tongued oligoleges in 187 pollen visits show red 13.3, white 19.2, yellow 67.3; in 109 nectar visits to unrelated flowers they show red 7.3, white 70.6, yellow 22.0.

In the case of the 235 plants observed before July, the colors are red 26.3, white 44.6, yellow 28.9; while of the 262 plants observed after June the colors are red 34.3, white 33.5, yellow 32.0.

Of 19 groups of bees whose visits were distributed under these two sets of flowers, 8 show a change of color in their maxima. Before July, 14 show maxima under white and 5 under red. After June, 8 show maxima under white, 6 under red, and 5 under yellow. The changes are determined by the composition of the flora, and have little to do with color preference. When the maxima change from other classes to B′, 47.1 per cent of whose flowers are yellow, there is a probability that some will fall under yellow.

According to LUBBOCK (**6**), the hive-bee prefers blue. When about three-fourths of the flowers whose nectar it can reach are white or yellow, how is a bee which flies all season and gathers so much honey going to limit itself to blue flowers? Of 196 local visits, 24.4 per cent are to red, 43.3 to white, and 32.1 to yellow.

It is an interesting fact that when bees become restricted to certain flowers, the preference is determined by the natural relationship of the flowers, and not by their colors. Of 83 local oligolectic bees, in spite of the fact that the restriction would be expected to limit the flowers to one color, 31 get their pollen from flowers of more than one color.

The first thing that the anthecologist learns is that the most important condition of insect visits is the presence of a supply of nectar and pollen, and that the most favorable time for observing insect visits is early in the morning, or soon after the flowers open. Bees clean up the pollen and nectar, and leave the flowers, which still remain as conspicuous as ever. In many flowers, like *Tradescantia* and *Desmodium*, the large bees are followed by small bees and Syrphidae in search of stray pollen.

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LITERATURE CITED

1. DARWIN, C., Fertilisation of orchids. 1862. 2d ed. 1877.
2. DELPINO, F., Ulteriori osservazioni sulla dicogamia nel regno vegetale. Atti Soc. Ital. Sci. Nat. **16**:151–349. 1873. (**2**²:1–201. 1875).
3. GRAY, A., Structural botany. 1879.
4. KERNER VON MARILAUN, A., The natural history of plants. **2**:1–983. 1894. Transl. Pflanzenleben. 1891.
5. KNUTH, P., Handbook of flower pollination. **1**:1–382. 1906. Transl. Handbuch der Blütenbiologie. 1898.

6. LUBBOCK, J., Ants, bees, and wasps. 1882. Review by ROMANES in Nature **26**:121–123.
7. LUTZ, F. E., Flowers and their insect visitors. Nat. Hist. **23**:125–134. 1923.
8. MÜLLER, H., The fertilisation of flowers. 1883. Transl. Befruchtung der Blumen. 1873.
9. ———, *Bombus mastrucatus*, ein Dysteleolog unter den alpinen Blumenbesuchern. Kosmos **5**:422–431. 1879.
10. ROBERTSON, C., Flowers and insects. Labiatae. Trans. Acad. Sci. St. Louis **6**:101–131. 1892.
11. ———, Flowers and insects. Ibid. **7**:151–179. 1896.
12. SCHULZ, A., Beiträge zur Kenntniss der Bestäubungseinrichtungen und Geschlechtsverteilung bei den Pflanzen. II. 1890.
13. SPRENGEL, C. K., Das Entdeckte Geheimniss der Natur im Bau und in der Befruchtung der Blumen. 1793.
14. TRELEASE, W., On the fertilization of several species of *Lobelia*. Amer. Nat. **13**:427–432. 1879.
15. WEED, C. M., Ten New England blossoms and their insect visitors. 1895.

CELLULAR STRUCTURE OF TENDRILS

HENRIETTA LISK

(WITH FIVE FIGURES)

Although the physiology of tendrils has been exhaustively studied, comparatively little work of an anatomical nature has been done. In reviewing the literature included in this paper, all references are to the structure of tendrils in general, unless specific examples are given.

PENHALLOW (**6**), in investigating the movements of *Cucurbita maxima* and *C. Pepo* tendrils, considered at some length their histological structure. In cross-sections of squash tendrils he found beneath the epidermis a somewhat thick layer of collenchyma, its continuity broken at three points by parenchyma tissue. The three parenchyma regions were found upon the upper side and the right and left. The cells of these regions were large, rounded, and somewhat thin-walled. They contained protoplasm and considerable chlorophyll. Within these areas were intercellular spaces with corresponding stomata in the epidermis. PENHALLOW found that in these parenchyma regions the greatest growth occurred without regular order, but in turn. He gave to this tissue the name vibrogen, signifying that the region of circumnutation was to be found there.

Within the layer of collenchyma were found three or four rows of rather large and rounded parenchyma cells. These cells were filled with "protoplasm, chlorophyll, and other granular matter." The amount of chlorophyll was much less than in the vibrogen region with which this tissue was connected. Beneath the parenchyma were found wood cells. In the early period of growth of the tendril, the wood tissue formed a crescent along the lower side. Later, breaks were found in the tissue corresponding to the position of the vibrogen regions. Finally these breaks were found to close up, and the tissue became a ring of wood. With age the wood cells developed thick walls. Next in the remaining tissue were found the fibrovascular bundles, seven in number. The central pith

was often seen to be shrunken, leaving the basal portion of the tendril hollow.

WORGITZKY (**8**), as a result of his investigations, came to the conclusion that the structure of tendrils stood in close relation to their function. As a demonstration of the fact that the main functions of tendrils were mechanical, he pointed out that a tendril which did not reach a support formed a spiral and died. As mechanical demands differed before and after the tendril surrounded a support, changes in structure were necessary. Differences in structure were found in the portion surrounding a support and the portion forming a spiral, for stiffness was necessary in the one, and elasticity in the other. Both portions were alike, however, in the unilateral development of the side which became attached, thus inducing dorsiventrality. Dorsiventrality, WORGITZKY found, was brought about in two ways: in the anlage, the anatomical structure was radial, and dorsiventrality appeared only when one-sided demands were made upon the curved portion of the tendril; or the arrangement of tissues in the very youngest stages was dorsiventral. The first was the case in tendrils showing secondary thickening, and here the dorsiventrality was brought about through the activity of the cambium. Where dorsiventrality was present in the very beginning, as in the majority of cases, WORGITZKY considered that this was due to the development of the tendril from a dorsiventral organ, as the blade of a leaf. He further concluded that the manner in which dorsiventrality was brought about differed in different tendrils, and in different regions of the same tendril. He found it true almost without exception that in the spirally twisted portion the mechanical elements lay on the concave side. The tissues of the concave side developed a cushion of tissue, which served to protect the tendril from the support, and at the same time brought about a closer union between tendril and support. The cells of the concave side were often stretched in a radial direction. This was conspicuous in the cortical parenchyma and collenchyma of the Cucurbitaceae.

Although the Cucurbitaceae were among these tendrils showing dorsiventrality in the earliest stages, they often showed a radial structure in the basal region. In these tendrils the dorsiventrality

was brought about primarily through the strands of mestome. The largest bundle lay on the concave side, and on either side, in a half moon shape, the smaller bundles were arranged. The bundles were almost always of an uneven number in the upper portion of the tendril (usually 5–7), and an even number in the base. The distribution of bast and collenchyma was found to follow that of the bundles. The changes which occurred after coiling around a support, and which made dorsiventrality more conspicuous, took place outside the bundles, and especially in the portion lying against the support. The indentation, which was found on the side becoming convex, flattened out as the tip was neared.

In the Cucurbitaceae, a peculiar forking of the bicollateral bundle, without cambium, was noticeable. The newly formed bundles finally weakened and disappeared, or united with others. The rule seemed to be for five bundles in the curved portion of the tendril, no matter how many at base, middle, or tip. The bast was found to form a complete ring in the basal portion of the tendril, or to have an opening on that side which in the upper region was to become convex. The complete ring was usually found in those tendrils which did not develop secondary thickening. In the Cucurbitaceae bast was often arranged in strands. In *Cucumis sativa* it was found with an opening of the ring on the convex side. Often transitional stages between true bast and true parenchyma (pith) were observed.

Worgitzky found collenchyma to be typical for most young tendrils; either present in a closed ring, or in parallel bands stretched tangentially rather than radially. From the fact that collenchyma was often in isolated strands in the basal portion, and in a continuous ring in the middle and tip portion, he concluded that the arrangement of collenchyma bore a relation to the mechanical function of the tendril, for a closed ring could not only withstand bending but torsion also. Collenchyma arranged in isolated bands was found only in those tendrils having angles, and only in these angles. Tendrils having an indentation on the side becoming convex had collenchyma strands on each side of the indentation. The number of strands was found to coincide with the number of large bundles or groups of bundles. While in the young tendril, the collenchyma

had great mechanical significance; it lost its importance after the tendril surrounded a support. The formation of the closed ring of xylem had now begun, and these cells took up the mechanical function of the tendril. In all cases where collenchyma was present, the cortical parenchyma had less chlorophyll and fewer intercellular spaces. The cells were long and prismatic, and arranged in rows. The nearer the tip, the more noticeable was the rarity of chlorophyll in the cortical parenchyma. He also noted that chlorophyll was found in quantity only when the parenchyma extended to the epidermis. Where epidermis and cortical parenchyma came in contact was often the only place where stomata were found in the epidermis. The pith parenchyma consisted of large cells with large intercellular spaces. In the region where the tendril attached itself to a support, the pith became thick walled.

MÜLLER (5), in his researches on the tendrils of Cucurbitaceae considered the relation between the behavior of tendrils and their anatomy. He concluded that curvature accompanied dorsiventrality. In support of this theory he noted the following points. The basal, insensitive portion of the tendril had central structure; the upper sensitive portion was bilateral. In the first the sclerenchyma was closed; in the second it was open. In the first the bundles were distributed equally on all sides; in the second they lay more and more on the concave. So far as the tendril had central structure it could not bend, but curved where it was bilateral. Sensitiveness increased toward the tip, as did the bilateral structure. The nearer the tip was approached, the more flattened was the structure, and the more narrow the sclerenchyma. Thinness of the tendril, thinness of cell walls, and transparency of cell walls were all factors which furthered sensitiveness.

In studying the arrangement of tissues in the tendril, MÜLLER pointed out that those structures easy of change were on the convex side, while the more resistant structures were on the concave. The sclerenchyma was on the concave side. The larger vascular bundle lay on the concave side, and the other bundles lay more and more on this side as they approached the tip. From base to tip of the tendril there was a broad strip of collenchyma on the concave side, while on the convex side there were found large thin-walled

parenchyma cells. The upper convex side, as most easily changed, could take up liquids, and become stretched. MÜLLER accordingly believed that his anatomical results showed that curvature was due to elongation of the convex surface, and not to contraction of the concave. As curvature followed only when stimulus was applied to the lower side, he suggested that the collenchyma must first take up the transmission of stimulus, then pass it to the parenchyma, either through the sclerenchyma or to the right and left of it. As the parenchyma was most subject to pressure it transmitted the stimulus.

MÜLLER found that true sclerenchyma was present in the bud in the tendrils of *Cucurbita* and *Bryonia*. In *Cyclanthera* and *Sicyos*, sclerenchyma was not observed until the tendrils became sensitive. So long as the tendrils were sensitive, the sclerenchyma was not lignified. When lignification set in, it began in the center of the concave side, and extended to the right and left. MÜLLER often found the lower sclerenchyma entirely lignified, while the upper showed no traces of lignification. He also noted that the xylem became lignified before the sclerenchyma, and that the sclerenchyma of the tendril became lignified before that of the stem adjoining.

In taking up the anatomical changes in that portion of the tendril which has surrounded a support, MÜLLER found that this region became swollen, hard, and brittle. The swelling was brought about by an enlargement of the cells of the epidermis, collenchyma, and the parenchyma lying between the sclerenchyma and the periphery. There was no cell division, but simply an increase in the size of the cells.

In comparing the hardening of the portion of the tendril which surrounds a support and that portion which forms a spiral, MÜLLER found that in the former the entire central parenchyma became lignified. The sclerenchyma formed a complete ring, the upper side of which was often found to be the thicker. This ring was the result of gradual sclerenchymatous thickening of parenchyma cells. On the lower side and on the right and left, the new sclerenchyma ring was bounded by the old sclerenchyma. On the upper side, however, the ring often lay directly beneath the epidermis. All tissues within

this ring were found to become lignified, with the exception of the phloem. The epidermis, collenchyma, and parenchyma, outside of the ring, usually did not become lignified. In the portion of the tendril which formed a spiral, only sclerenchyma and xylem became lignified, consequently the tendril remained elastic at this point.

Among the Cucurbitaceae which MÜLLER took up specifically was *Cyclanthera pedata*. Of these tendrils, he studied both branched and simple. In the branched tendrils he noted the following characteristics: The main axis was usually six-sided, but not entirely central in structure. Two of the angles were much smaller than the other four. The collenchyma lay regularly on the outer edges of the angles. The layer of chlorophyll cells contained much of this substance. The sclerenchyma described six curves, and in each arc lay a bundle, the two smallest bundles lying in the smallest arcs. The four largest bundles were arranged in the form of a right angled cross. Between the six bundles in the basal portion of the tendril were seen six smaller bundles of few cells. These disappeared toward the tip.

The branch of the tendrils studied was found to have five angles, the lowest of which was the largest. Collenchyma lay in the angles. The sclerenchyma was open on the upper side, and described five arcs corresponding to the angles. Five bundles lay in the angles, and sometimes a sixth appeared in the opening of the sclerenchyma. As the tip was approached the largest angle became more and more flattened, until the circumference of the cross-section was almost square. The collenchyma remained until very near the tip. Openings appeared in the sides of the sclerenchyma ring, and the original upper opening became larger. The arcs finally disappeared toward the tip.

A cross-section of the base of a simple tendril showed a four-angled structure with collenchyma in the corners. Next lay the parenchyma cells containing chlorophyll, and a closed ring of sclerenchyma corresponding in shape to the circumference of the tendril. The four bundles lying in the angles were usually nearly the same size, with sometimes the lower the largest, and the upper the smallest. In the sensitive portion of the tendril, the upper

bundle broadened and became heart-shaped. From this two bundles were formed. As these bundles separated, the angle in which they lay became broader. The collenchyma split in two parts, and an indentation was formed between these parts, which became deeper as the circumference of the tendril became five-angled instead of four. The sclerenchyma also split between the two newly formed angles. The cross-section of the tip of a simple tendril was formed to resemble that of the base of a tendril branch.

MACDOUGAL (3), working with the tendrils of *Passiflora caerulea*, had for his purpose the determining of "the factors in the movements of the tendrils of the Passiflorae, more particularly the movements by which a tendril responds to a stimulus, resumes its original position, or on continuance of the irritation coils permanently." He also investigated the subsequent changes in the tendrils while coiled and serving as a support for the adjacent parts of the plant. To determine the conditions prevailing in the tendril during the sensitive period, MACDOUGAL studied the development through all stages of growth from the time of the appearance of the tendril as an axillary papilla until it passed the sensitive stage. The greatest difficulty was encountered in fixing and hardening the material without causing the tendril to roll up in a helix, distorting the wall and contents of the cells. After a wide range of experiments, the best results were obtained by the use of acetic alcohol (1 part glacial acetic acid, 6 parts absolute alcohol [or 96 per cent], and 3 parts chloroform). In this killing agent the tendril, after two or three oscillations, regained and kept almost the original form.

MACDOUGAL described the tendrils of *Passiflora caerulea* as filamentous organs springing from the axils of the leaves. In tendrils 1–3 cm. in length the whole surface often had a reddish purple tinge, due to color bodies in the subepidermal cells. With growth the color became distributed in ill defined longitudinal bands. When a tendril had reached the coiling stage, three distinct regions were to be found, the base, or non-coiling part 3–4 cm.; the middle or coiling region, comprising the greater part of the organ; and the hooked tip 4–6 mm. in length. The whole organ showed a bilateral organization, which was least apparent at the base, and most pronounced in the portion having the greatest power of movement.

The basal portion of the tendril was broadly oval, slightly flattened on the concave side; the middle portion was oval, with the lateral diameter much greater than the transverse, and with the lower surface distinctly flattened; the tip was nearly circular in outline and bore at the extreme end a cup-shaped depression, the result of excessive growth in length of the periblem. Along the convex upper and the lateral sides of the tendril were obscure angles, which were usually absent from the concave side.

Sections through the middle portion of the tendril showed the following structure: The epidermis consisted of a layer of rectangular cells with stomata on both surfaces. The layer of collenchyma was found to have the tangential walls much thicker than the radial. At the obtuse angles this layer consisted of three cells; at other places it had one. In this tissue were found the color bodies. The parenchyma consisted of loosely arranged, thin-walled cells containing much chlorophyll and protoplasm in the outer row. The inner row of cells was heavily laden with starch. Crystals of calcium oxalate were found throughout the parenchyma. The cells of this layer on the convex side of the tendril were larger than on the concave surface. The intercellular spaces were large and many. In many cases, however, the entire ends of adjacent cells were pressed closely together, so that one cell sent a protrusion into the cavity of another. The structure and arrangement of these cells fitted them for great variation in size, while the intercellular spaces, affording space for the reception of expelled cell sap, made possible rapid changes in tension. The parenchyma was connected with the central pith by medullary rays 2–4 cells in height. The bast region consisted of thin-walled, closely packed cells containing much protoplasm. As the tendril approached maturity these cells became less active, and took on excessive wall thickenings. When the tendril was still very young, this tissue formed a continuous band interrupted only by the medullary rays. The cambium appeared about the time of maturity, and retained its activity even after coiling had taken place. The primary xylem elements were about ten; half in a nearly straight row across the concave side, and the remainder in a semicircle to conform to the outline of the convex side. Each bundle consisted of two or three

spiral vessels arranged radially. These vessels showed lignification even in the young tendril. A continuous band of wood was found on the concave side by the formation of the secondary bundles. The xylem elements of the convex side did not change until after coiling. The central pith was composed of large parenchyma cells containing protoplasm.

The basal portion of the tendril differed from the middle portion in its regular oval outline, symmetrical arrangement of the xylem, heavier thickening of the collenchyma, and the early formation of a continuous distinct cambium zone. Lignification had extended slightly to the pith and parenchyma in the xylem, which had three or four spiral vessels beside an annular vessel in each bundle. The central pith was usually torn apart. The structure of the tip differed widely from the base or middle region. The cup formation lay transversely to the length of the tendril near the extremity of the concave side. The epidermal cells were smaller toward the end of the tendril. The collenchyma was composed of one row of shortened, thickened cells terminating at the rim of the cup. The chlorophyll layer was reduced in size, and showed an increased density in protoplasm. The parenchyma layer was relatively very thick. The cells were angular and distorted. The bast and cambium disappeared after they entered this region. The bundles of the convex side and the band of the concave side were separated by a thin bundle of pith as they neared the tip. Pits were present in all the tissues of the tendril. The protoplasm was most dense and granular in the epidermal and chlorophyllous cells of the concave side near the tip. Density of protoplasm decreased in the middle region.

MacDougal assumed in conclusion (1) that the concentration of the protoplasm in the epidermal layer had a direct connection with irritability; (2) that the movements of the organs were due to changes in the chlorophyll layer; (3) that the disposition of the xylem elements was favorable to rapid flexion and extension; and (4) that the abundant food supply reserve was a provision for the rapid growth and fixation of the tendril upon coiling.

Later MacDougal (4) added a few points to the structure of *Passiflora* tendrils which were not given in the earlier paper.

He described the epidermal cells as rectangular in outline, with the long axis parallel to the long axis of the tendril. The protoplasts were found to occupy a large proportion of the cell cavity. The nucleus, especially on the concave side, usually lay against the inner wall. The structure of the protoplasm of the convex and concave sides was quite different, that of the concave side being more richly granular, and more nearly filling the cell cavity. The density of the protoplasm of the concave side increased from base to tip, apparently corresponding with the degree of irritability to contact. The outer walls of the concave side were slightly arched outwardly, and became cuticularized only after maturity. These convexities, MACDOUGAL suggested, must greatly increase the delicacy of perception of stimuli.

The epidermal layer contained stomata which communicated with the cortical parenchyma by very narrow intercellular passages. The collenchyma layer was one, two, or three cells in thickness. The protoplasm of the concave side almost completely filled the cells, and was densely granular, in marked contrast with that of the convex side. MACDOUGAL remarked that this difference between the contents of the collenchymatous tissue of the two sides was an indication that the densely granular condition of the concave side bore a connection with the transmission of impulses transversely or longitudinally. The granular contents, however, might be present merely as reserve substance.

In comparing the parenchyma cells internal to the collenchyma, MACDOUGAL found differences in their form and behavior in the convex and concave sides. The cells of the concave side were longer in proportion to their width than those of the convex side. The cells of the concave side had tapering ends, and the end walls were united over a small area only, thus forming large intercellular spaces. The cells of the convex side were united more completely across the ends, and the intercellular spaces were accordingly smaller. The protoplasm of the parenchyma cells also was more densely granular on the concave side, and more nearly filled the cell cavity. The parenchymatous cells were arranged in four or five layers on both the concave and convex sides of the tendril,

but those of the convex had the greater diameter, thus giving this side preponderance in thickness. In the two outer layers were chloroplasts varying from six to ten in number. The parenchymatous tissue of both cortex and pith exhibited marked infoldings of the walls. During curvature MACDOUGAL found that the cells of the parenchyma on the concave side decreased in size from 20 to 40 per cent of their original volume, and became irregularly globoid or ovoid. This MACDOUGAL thought to be explained by "an increase in the permeability of their protoplasts, a consequent extrusion of water into the intercellular spaces, and a release of the stretching tension of their walls, the elastic contraction of which caused the resultant curvature."

HABERLANDT (2) observed that in those tendrils which were sensitive on one side only, the sensitive side was usually without hairs and had few if any stomata. He noted that the sensitive epidermis of tendrils differed from other epidermis in various points. The epidermal cells were usually smaller but deeper. The outer walls were scarcely cutinized at first, but after the tendril had attached itself the walls became thick and heavily cutinized. The true cuticle was usually well developed and folded, although sometimes it was absent at the base of the tendril. The folding of the cuticle was much more pronounced in the sensitive portions of the tendrils of Passiflorae and others than it was on the stem and petiole of the same plants. He considered this folding of the cuticle to be an adaptation for the perception of stimulus. In most tendrils the outer walls of the sensitive epidermal cells curve outward, another useful adaptation. The epidermal cells were rich in cytoplasm, a characteristic of sense organs. HABERLANDT stated that PFEFFER (7) was the first to call attention to the sensitive papillae of *Cucumis sativus*, *Bryonia dioica*, and *Sicyos angulatus*. In *Cucumis* the papillae were observed only on the sensitive side of the tendril; in *Bryonia* they were found on both sides. In both plants, however, the papillae were almost completely absent from the basal, insensitive portion. PFEFFER called attention to the bowl-like widening of these points, so that the sensitive protoplasm was spread over an increased surface. As he did not find similar

papillae in all plants, he concluded that they were not absolutely essential for the perception of stimulus, but merely facilitated this perception.

HABERLANDT found great variety in the structure of these papillae. In *Cucurbita Pepo* the "sense epithelium" of the sensitive side lay above a continuous wide band of collenchyma. Stomata were absent, but a few club-shaped hairs were observed. The sense cells were somewhat stretched, the outer walls being 3–4 μ thick. On the center of each outer wall there was one papilla, with very rarely a second found near it. The interior of the papilla was widened in a funnel-shaped fashion, and was approximately circular in circumference, 1.5–6 μ in diameter. The canal leading from the inside of the papilla into the lumen of the cell was oval, or even slitlike in cross-section. The thin outer membrane of the papilla was flat, or sometimes very slightly bulged outward, and 0.6–0.8 μ in thickness. The thickness of the cuticle at this point was practically the same as at other regions in the tendril. Beneath it lay a very thin cellulose lamella. The sense cells contained a thick layer of protoplasm directly beneath the cell wall, in which were chromatophores containing starch, and a large nucleus against the inner wall. The papillae were usually completely filled with cytoplasm. If they were large, however, the cytoplasm seemed to lie against the wall. A crystal (probably calcium oxalate) usually lay in the protoplasm of the papilla. Sometimes the crystal was found in the opening of the canal, or in other positions. HABERLANDT offered no evidence as to the origin of this crystal (rarely more than one), but suggested that it bore a close relation to the perception of stimulus. A sudden pressure, bringing the sensitive membrane of the cell in contact with a corner of this crystal, might produce a stronger stimulus.

In *Lagenaria vulgaris*, as in *Cucurbita Pepo*, papillae were present only on the sensitive side. In shape the papillae much resembled those of *C. Pepo*. In the tendrils of *Cyclanthera explodens* the papillae were present only on the sensitive side. HABERLANDT described them here as being a "circular flat depression, easily overlooked." The outer wall of the cell was 1.5–2 μ in thickness, and the membrane over the papilla was 0.8 μ thick. The membrane showed a weak bowing outward.

BRUSH (1), in determining the influence of tension and contact upon the formation of mechanical tissue in the tendrils of *Passiflora caerulea*, used two methods of comparison, that is, their breaking strength and their anatomical structure. In studying the general anatomy of *Passiflora caerulea* tendrils, he found the following arrangement of tissues: epidermis, collenchyma, thin walled parenchyma, bast, xylem (which forms a complete ring due to secondary growth), and pith. In mature tendrils the pith filled the central part except in the basal portion of the tendril, where there was a central cavity.

At the base the arrangement of tissues was found to be very nearly radial, but in the "portion in contact" dorsiventrality was marked. A section halfway between base and apex showed a slight dorsiventrality, a somewhat greater amount of xylem being formed on the concave side.

The mechanical tissue of a free tendril, as shown by cross-sections through the middle of the tendril, was limited to a small area of xylem on the concave side, and the four primary cells were comparatively thin walled, and the primary bundles of the opposite side were composed of two or three slightly thickened vessels. Only a few bast fibers were present, and a very little pith, which lined a central cavity. Toward the base a complete ring of thin walled xylem and pith was formed.

A comparison of sections of tendrils under contact and under tension showed that while the xylem areas were approximately the same in both, in the tendril under tension the walls had become much thickened in the pith cells, while in the one under contact alone the pith cells were quite thin walled. This thickening of walls took place throughout the whole area of the pith in the tendril under tension. The pith was thin walled throughout in the tendril under contact alone. BRUSH noted that those tendrils under tension were harder to cut, owing no doubt to a difference in density of cell walls. An examination of sections of the middle third of tendrils with and without tension showed the diameter of mechanical tissue to be greater in the latter. BRUSH, having found in experiments on the breaking strength of tendrils that those under tension had a breaking strength nearly 50 per cent higher (average under tension 1239 gm., average tension-free 862 gm.), found the greater

area of mechanical tissue in the tendril grasping a support but not under tension somewhat surprising. He thinks the increased strength with tension partly accounted for by the thickening of the pith cell walls, this thickening usually extending to the center. Measurements with a planimeter showed the comparative areas to be as follows:

	Xylem	Pith (thick walled)	Total
Under tension	2.71	2.78	5.39
Tension free	4.51	2.78	4.51

Brush found that a marked characteristic of sections of tension free tendrils was that the pith became displaced in sectioning, while in those from tendrils under tension the pith held its shape as if firm.

Sections made from the basal part of tension-free tendrils and those under tension showed remarkably the transference of stimulus for growth through the tissues to the basal portion of the tendril. In the base of a tendril in which the middle third only had been under tension, the pith was thick walled throughout, while in the base of a tension-free tendril the pith was thin walled. Brush states that the marked increase in the thickness of pith walls which was found only when tension was present can be explained only by the theory that this thickening is due to the longitudinal pull on the tendril, by which the tensile strength of the tendril is increased.

Examination of sections through the contact region of tendrils which had been placed under pressure by a column of mercury failed to show any difference in anatomical structure when compared with tendrils in contact only. Sections through the middle of tendrils not under tension, but placed under pressure and under contact, were markedly different in their amount of xylem. The area of xylem in an average tendril under pressure was approximately twice as great as in a tendril which had been in contact only. The pith was thin walled in both sets of tendrils. Brush concludes from his anatomical studies on *Passiflora* "that contact pressure causes a greater formation of xylem in the tendril, while longitudinal tension causes a thickening of the walls of the pith whereby greater tensile strength is secured."

A study of the cellular structure of the tendrils of *Cyclanthera explodens* has been carried on by the writer. Thirty-three free,

uncoiled, and (so far as possible) unstimulated tendrils of *Cyclanthera explodens*, varying in length from 1 cm. to 12.6 cm., were studied cytologically from stained preparations. These tendrils were sectioned throughout their entire length. Freehand sections from living material also were studied.

Great difficulty was at first experienced in fixing those tendrils which had reached the sensitive stage, but their reaction to the killing agent was practically overcome, in the great majority of cases, by inserting the tendrils in glass tubes of small bore immersed in the solution. Fleming, weak, medium, and strong, and Picro formin were the killing agents used. Triple Fleming was used in staining.

Cross-sections of the basal portion of a sensitive tendril (a tendril of at least 6 cm. in length) were markedly radial in structure. Six well defined lobes were frequently observed, four large and two very much smaller. One or both of the latter were often missing, but if present were found on the dorsal surface of the tendril. The number of lobes always corresponded to the number of bundles found in this region. The epidermis was protected by a thick cutin, which rendered the epidermal cells difficult to cut without becoming torn. The epidermal cells were rectangular in shape, with their depth greater than their width. The outer walls curved outward in all cases, and were frequently extended to form "papillae." These papillae were more numerous, however, in the basal portion of the tendril than in the sensitive region. They were usually found upon the outermost projection of the lobes, one to three in number, with often two papillae lying in adjacent cells. The "sense cells" were more narrow than the ordinary epidermal cell, and, as HABERLANDT stated, had large nuclei which lay on or near the inner wall. The cytoplasm extended into the papilla, usually filling it.

The epidermis of this region, as of all other portions of both sensitive and insensitive tendrils, was found to be more or less plentifully provided with glandular structures, usually club-shaped, but with all variations in size and shape. These epidermal outgrowths were sometimes found as an enlarged papilla-like structure into which the cell contents had entered. This cell often divided,

and the process of division continued until the filament was three or four cells in length (fig. 1). In other instances, the outgrowth was plainly separated from the epidermal cell by the cell wall. Crystals were common in these bodies, especially after the connection with the tendril had disappeared. Crystals were also found in the parenchyma of the tendril. The collenchyma of the basal region was found only in the angles of the lobes. This layer was composed of 3–5 rows of cells having the tangential walls heavier than the radial. These rows of cells were usually deeper in the ventral, and right and left lobes than upon the dorsal surface. Frequently the inner row appeared to be a transition between collenchyma and parenchyma. The cortical parenchyma was 2 or 3 rows in depth, the inner row being composed of larger cells, and containing starch in abundance. The starch content was especially heavy in those cells lying in the outermost projection of the lobes, and at the lowest point in the curves between the lobes. The closed ring of bast, composed of very much elongated cells, densely filled with cytoplasm, conformed in shape to the outline of the cross-section of the tendril. The bundles, as before stated, corresponded in number to the lobes. The largest bundle lay on the ventral lobe. The pith parenchyma consisted of large, thin walled cells, very irregular in size and shape, but closely interlocked with one another. The intercellular spaces were small.

A cross-section through the middle portion of any tendril showed it to be dorsiventral in structure. The large lobe of the dorsal surface had given place to an indentation on this side (fig. 2). The bast layer now extended across the concave side only, with two small strips lying outside the small bundles of the convex (dorsal) surface. The number of bundles throughout the dorsiventral portion of a tendril was commonly five. An exception to this was noted in a specimen in which the branch and main axis of the tendril were united until within a short distance of the tip. Here the bundles were eight in number, although the structure was dorsiventral.

Gradually the central lobe of the concave side disappeared, and the outline of this surface was found to be broadly semicircular in the sensitive region of a tendril (fig. 3). The collenchyma layer of the concave side in this region consisted of apparently one row

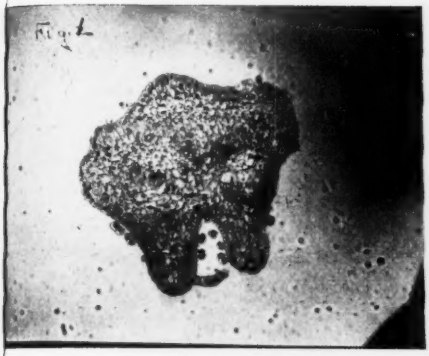

FIG. 1

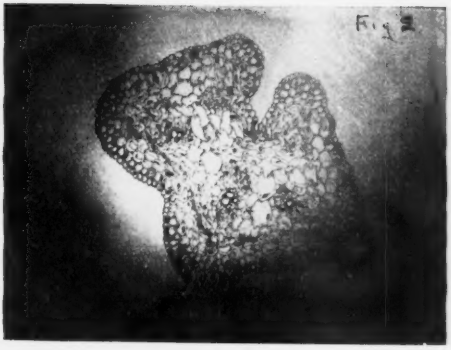

FIG. 2

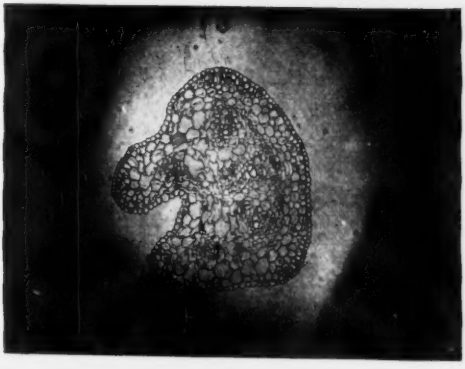

FIG. 3

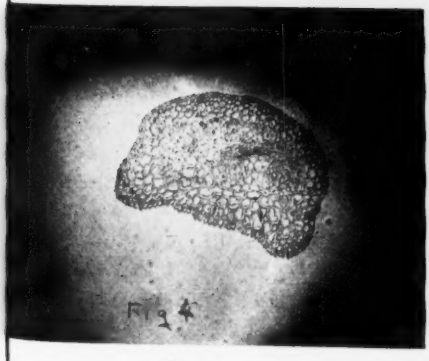

FIG. 4

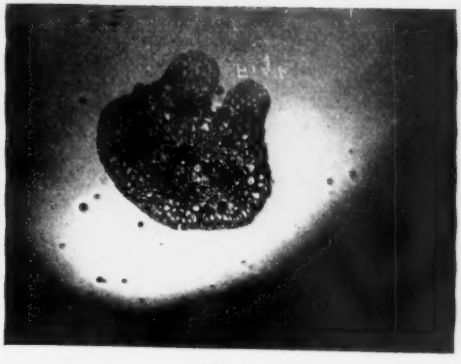

FIG. 5

of cells, whose walls showed a tendency to break down, often throwing four or five cells together. This was much more marked upon the concave side than on the convex, but, as this breaking down had been observed in a slight degree in the basal portion of the tendril, it was of doubtful significance. The parenchyma cells of the convex side were now seen to be much larger than those of the concave. Both cortical and pith parenchyma contained large intercellular spaces.

The indentation upon the convex side now widened until a cross-section nearing the tip of the tendril became semicircular, or almost rectangular in outline (fig. 4). The collenchyma of the concave side consisted of three rows of cells, the outer row being of very small cells of practically the same size and appearance as the epidermal cells. The pith parenchyma had now become greatly compressed, the bundles filling the greater part of the central space. A cross-section near the base of a young insensitive tendril (2–5 cm. in length) was found to be dorsiventral (figs. 1–5). The chief differences in structure which distinguished the young tendril from the upper portion of one which had reached the sensitive stage were the absence of strengthening tissue, the greater density of the cytoplasm, the presence of starch in greater quantity, and the large number and size of the nuclei.

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LITERATURE CITED

1. BRUSH, W. D., The formation of mechanical tissue in the tendrils of *Passiflora caerulea* as influenced by tension and contact. BOT. GAZ. **53**:453–476. 1912.
2. HABERLANDT, G., Sinnesorgane in Pflanzenreich zur Perception Mechanischer Reize. 117–139. 1901
3. MACDOUGAL, D. T., Mechanism of curvature of tendrils. Ann. Botany, **10**:373–402. 1892.
4. ———, The tendrils of *Passiflora caerulea*, anatomy and morphology. BOT. GAZ. **17**:205–212. 1896.
5. MÜLLER, O., Untersuchungen über die Ranken der Cucurbitaceen. Cohn Beiträge Biol. Pflanzen **4**:97–143. 1887.
6. PENHALLOW, D. P., Tendril movements in *Cucurbita maxima* and *C. Pepo*. Amer. Jour. Sci. and Arts **31**:49. 1886.
7. PFEFFER, Zur Kenntniss der Contactreize.
8. WORGITZKY, G., Vergleichende Anatomie der Ranken. Flora **70**:2, 17, 33, 49, 65, 86. 1887.

NITROGEN CONTENT OF WEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 321

E. G. CAMPBELL

(WITH FOUR FIGURES)

Several years ago Woo[1] made a chemical analysis of *Amaranthus retroflexus*. His work included these three stages of development: (1) young plants (collected June 5, one-fourth inch in height); (2) plants just before blooming (collected July 8, 20 inches in height); and (3) an intervening age (collected June 20, 6–8 inches in height). The purpose of his work in part was to investigate the claim, often made, that *Amaranthus* has the power to store nitrates. He found the largest percentage of total and nitrate nitrogen in the stage of growth just before blooming, but was unable to extend his investigation through the entire season, to include mature plants. In 1920 the writer, under the direction of Dr. WILLIAM CROCKER, undertook to investigate the nitrate nitrogen situation in *A. retroflexus*, including two ages, just before blooming and at maturity. These two growth periods were selected so as to check the work done by Woo and extend the investigation to the mature plant. Later the work was extended through 1921 and 1922, to include, in addition to *A. retroflexus*, twenty-five other weed species collected in three stages of development and under various conditions of growth.

Methods and results

In collecting specimens, care was taken that the three desired ages of any given species were secured from the same plot of soil, so as to insure so far as possible like conditions for the three stages of growth. The specimens were carefully cleaned in running tap water, oven dried, and ground to a fine powder. The powder was then brought to a constant dry weight, 2 gm. then being taken for the total nitrogen and 10 gm. for the nitrate nitrogen determination.

[1] Woo, M. L., Chemical constituents of *Amaranthus retroflexus*. BOT. GAZ. 68:313–344. 1919.

In all cases duplicate samples were run. In the work of 1920 and 1921 analyses were made of the separate parts of each specimen (root, stem, leaves, and fruit), but in the work of 1922 the entire plant of each growth stage was analyzed. The fall and winter conditions of several species were investigated and compared. In the case of *Amaranthus retroflexus*, specimens growing under abnormal conditions (on manure piles and dumps of hog hair) as well as field specimens were analyzed.

The Kjeldahl-Gunning method was followed in finding the percentage of total nitrogen. For the nitrate nitrogen determina-

TABLE I

NITRATE NITROGEN IN THREE AGES OF *Amaranthus retroflexus* AS DETERMINED BY TWO METHODS

STAGE	PART	SCHLÖSING-WAGNER METHOD		PART	PHENOLDISULPHONIC ACID METHOD	
		Nitrate nitrogen			Nitrate nitrogen	
		I	II		I	II
Young	Root	0.74	0.78	Root	0.64	0.59
	Stem	0.84	0.86	Stem	0.75	0.78
	Leaves	0.64	0.65	Leaves	0.45	0.48
Just before blooming	Root	1.02	0.99	Root	0.92	0.95
	Stem	1.84	1.89	Stem	1.40	1.45
	Leaves	Trace	.....	Leaves	0.25	0.28
Mature	Root	Trace	.....	Root	0.00	0.00
	Stem	Trace	.....	Stem	0.00	0.00
	Leaves	0.00	0.00	Leaves	0.00	0.00
	Fruit	0.00	0.00	Fruit	0.00	0.00

tion in *A. retroflexus*, both the Schlösing-Wagner volumetric method as modified by KOCH, and the phenoldisulphonic acid colorimetric method were employed. In all other species involved, the nitrates were determined by the phenoldisulphonic acid method alone. After paralleling the two methods in a great number of test analyses, the writer prefers the latter, because the apparatus involved is more easily and accurately operated. Of the two methods employed (volumetric and colorimetric) in the nitrate nitrogen determination on *A. retroflexus*, a higher percentage was repeatedly obtained by the volumetric method. This was the one used exclusively by WOO, who found most nitrate nitrogen in

the stage of growth designated in this paper as just before blooming. So far as the writer used the volumetric method, he obtained results closely approximating and consistently paralleling those of

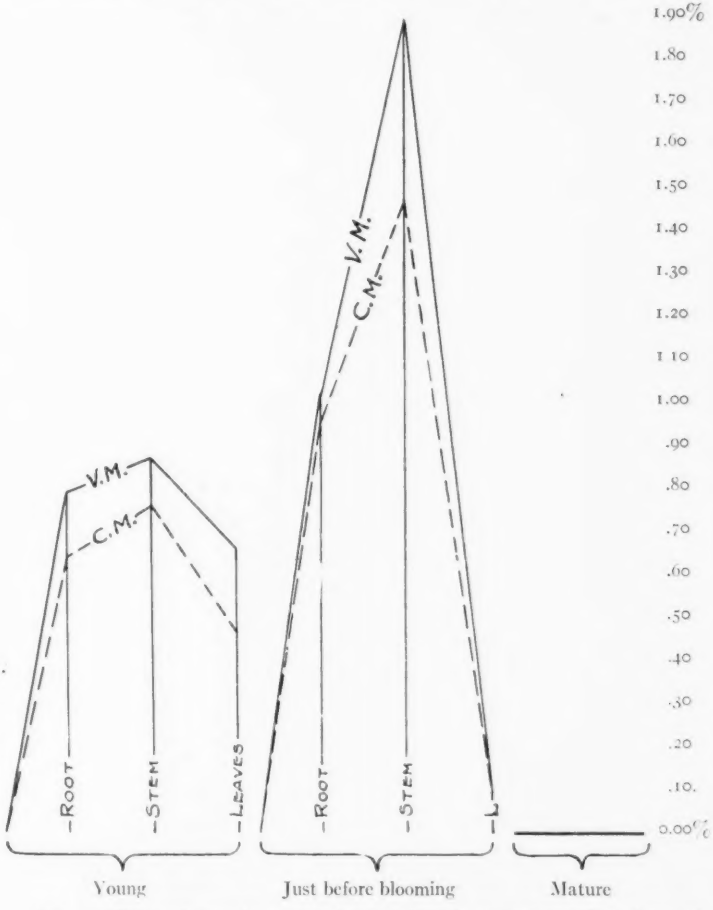

FIG. 1.—Nitrate nitrogen in *Amaranthus retroflexus*, in three stages of development grown under field conditions, as determined by two methods: V.M., volumetric (Schlösing-Wagner); C.M., colorimetric (phenoldisulphonic).

Woo. The results reached through the colorimetric method, however, ran slightly and persistently lower, as shown in table I and fig. 1.

The work of 1920 included only four species, *A. retroflexus* in two growth stages (just before blooming and mature), and the

TABLE II

NITRATE NITROGEN; VOLUMETRIC METHOD, 1920

Stage	Part	I	II
Amaranthus retroflexus (just before blooming)	Root	0.43	0.43
	Stem	0.31	0.30
	Leaves	0.19	0.18
Amaranthus retroflexus (mature)	Root	0.00	0.00
	Stem	Trace	.....
	Leaves	0.00	0.00
	Fruit	0.00	0.00
Abutilon Theophrasti (mature)	Root	0.00	0.00
	Stem	0.00	0.00
Erigeron canadensis (mature)	Root	0.00	0.00
	Stem	0.00	0.00
Xanthium italicum (mature)	Root	0.00	0.00
	Stem	0.00	0.00

TABLE III

RATIO BETWEEN TOTAL NITROGEN AND NITRATE NITROGEN IN THREE AGES OF *Amaranthus retroflexus*

STAGE	PART	TOTAL NITROGEN		NITRATE NITROGEN	
		I	II	I	II
Young	Root	3.96	3.96	0.64	0.59
	Stem	2.92	2.94	0.75	0.78
	Leaves	4.63	4.53	0.45	0.48
Just before blooming	Root	3.12	3.90	0.92	0.95
	Stem	3.58	3.71	1.40	1.45
	Leaves	4.59	4.55	0.25	0.28
Mature	Root	0.63	0.67	0.00	0.00
	Stem	0.24	0.28	0.00	0.00
	Leaves	1.99	2.00	0.00	0.00
	Fruit	2.58	2.66	0.00	0.00

root and stem of *Abutilon Theophrasti*, *Erigeron canadensis*, and *Xanthium italicum* in the mature stage only. None of these four species contained nitrates at maturity except *A. retroflexus*, which showed a trace in the stem (table II). In the stage just before

TABLE IV

Stage	Part	Total nitrogen		Nitrate nitrogen	
		I	II	I	II
Chenopodium album					
Young	Root	1.18	1.21	0.10	0.09
	Stem	2.56	2.57	0.28	0.24
	Leaves	5.09	5.12	0.08	0.12
Just before blooming	Root	0.89	0.95	0.32	0.32
	Stem	1.27	1.29	0.10	0.09
	Leaves	4.63	4.69	Trace	.....
Mature	Root	0.52	0.54	0.00	0.00
	Stem	0.63	0.67	0.00	0.00
	Leaves	3.96	3.96	0.00	0.00
	Fruit	3.11	3.15	0.00	0.00
Xanthium italicum					
Young	Root	0.81	0.77	0.16	0.15
	Stem	1.10	1.06	0.20	0.21
	Leaves	4.52	4.48	0.20	0.24
Just before blooming	Root	1.54	1.54	0.12	0.14
	Stem	2.19	2.20	0.25	0.27
	Leaves	4.50	4.48	0.12	0.14
Mature	Root	0.70	0.68	0.00	0.00
	Stem	0.29	0.29	0.00	0.00
	Leaves	1.96	1.96	0.00	0.00
	Fruit	1.79	1.79	0.00	0.00
Lactuca scariola					
Young	Root	1.54	1.54	Trace	.....
	Stemless	.....	.....	.....	.....
	Leaves	3.88	4.00	Trace	.....
Just before blooming	Root	0.77	0.70	Trace	.....
	Stem	0.77	0.79	Trace	.....
	Leaves	3.35	3.31	Trace	.....
Mature	Root	0.51	0.52	0.00	0.00
	Stem	0.35	0.37	0.00	0.00
	Leaves	1.37	1.40	0.00	0.00
	Fruit	2.45	2.43	0.00	0.00
Ambrosia artemisiifolia					
Young	Root	0.96	0.96	Trace	.....
	Stem	1.59	1.59	0.12	0.14
	Leaves	4.56	4.53	0.00	0.00
Just before blooming	Root	0.50	0.50	0.00	0.00
	Stem	0.57	0.57	0.00	0.00
	Leaves	3.59	3.37	0.00	0.00
Mature	Root	0.35	0.32	0.00	0.00
	Stem	0.25	0.27	0.00	0.00
	Leaves	2.10	2.14	0.00	0.00
	Fruit	2.06	2.04	0.00	0.00

TABLE IV—*Continued*

Stage	Part	Total nitrogen		Nitrate nitrogen	
		I	II	I	II
Erigeron canadensis					
Young	Root	0.61	0.69	0.00	0.00
	Stem	0.98	1.06	0.00	0.00
	Leaves	3.43	.....	0.00	0.00
Just before blooming	Root	0.35	.....	0.00	0.00
	Stem	0.38	0.35	0.00	0.00
	Leaves	3.43	3.45	0.00	0.00
Mature	Root	0.29	0.29	0.00	0.00
	Stem	0.35	0.35	0.00	0.00
	Leaves	2.65	2.67	0.00	0.00
	Fruit	1.95	2.07	0.00	0.00
Abutilon Theophrasti					
Young	Root	0.77	0.75	0.00	0.00
	Stem	0.69	0.71	0.00	0.00
	Leaves	3.22	3.20	0.00	0.00
Just before blooming	Root	0.63	0.67	0.00	0.00
	Stem	0.41	0.37	0.00	0.00
	Leaves	2.56	2.52	0.00	0.00
Mature	Root	0.32	0.32	0.00	0.00
	Stem	0.36	0.39	0.00	0.00
	Leaves	1.67	1.65	0.00	0.00
	Fruit	2.26	2.26	0.00	0.00

blooming the results reached closely approximate Woo's figures for root, stem, and leaves.

In the work of 1921, *Amaranthus* in three stages of its development (young, just before blooming, and mature) was analyzed for both total and nitrate nitrogen (table III).

During the same season six other species were tested with *Amaranthus retroflexus*, namely, *Chenopodium album*, *Xanthium italicum*, *Lactuca scariola*, *Ambrosia artemsiifolia*, *Erigeron canadensis*, and *Abutilon Theophrasti*. The results obtained in these additional species are shown in table IV.

The work of 1922 includes *Amaranthus retroflexus* growing under abnormal conditions, and eighteen other species of weeds collected in three stages of development and under various conditions of growth. The species are as follows: *Rumex crispus*, *Erigeron annus*, *Potentilla monspeliensis*, *Capsella Bursa-pastoris*, *Veronica*

peregrina, *Amaranthus blitoides*, *A. graecizans*, *Atriplex patula*, *Salsola Kali*, *Polygonum pennsylvanicum*, *P. convolvulus*, *Portulaca oleracea*, *Daucus carota*, *Arctium minus*, *Solanum carolinense*, *Cirsium arvense*, *Physalis heterophylla*, and *Asclepias syriaca*. In this group of plants no attempt was made to divide the specimens into root, stem, leaves, and fruit; the whole plant of each growth stage was analyzed.

In the spring of 1922, rye and five contemporary winter annual weeds were compared in three stages of growth. Rye was collected

TABLE V

WEEDS GROWING WITH RYE, COLLECTED IN THREE GROWTH PERIODS

I WHEN RYE WAS 2–3 INCHES HIGH

II WHEN RYE WAS BEGINNING TO JOINT

III WHEN RYE WAS IN FULL HEAD

Plant and growth period	Total nitrogen	Nitrate nitrogen	Plant and growth period	Total nitrogen	Nitrate nitrogen
Rye			Veronica peregrina		
I	3.78	0.08	I	2.38	0.04
II	2.11	0.05	II	1.40	0.04
III	1.19	0.02	III	1.20	0.04
Rumex crispus			Erigeron annuus		
I	3.22	0.10	I	3.36	0.08
II	2.38	0.04	II	1.68	0.04
III	1.96	0.04	III	1.13	0.04
Capsella Bursa-pastoris			Lactuca scariola		
I	4.62	0.15	I	3.36	0.08
II	3.05	0.08	II	1.90	0.04
III	1.54	0.02	III	1.78	0.04
Potentilla monspeliensis					
I	3.36	0.12			
II	1.68	0.04			
III	1.14	0.04			

when from two to three inches high, when beginning to joint, and when in about full head. *Capsella Bursa-pastoris*, *Erigeron annuus*, *Lactuca scariola*, *Potentilla monspeliensis*, and *Rumex crispus* were each collected on the same days and in the corresponding stages of growth as the five rye collections. The results of this investigation are shown in table V. It is interesting to note that there is a close parallel between rye and weeds, both in total and nitrate nitrogen, and that throughout the series the curve goes

TABLE VI

Stage	Percentage total nitrogen	Percentage nitrate nitrogen	Lb. of nitrate in soils per acre
Polygonum pennsylvanicum			
Young	1.42	Trace	.....
Just before blooming	3.38	0.1	3
Early maturity	0.55	0.00	8
Polygonum convolvulus			
Young	3.57	0.06	.....
Just before blooming	3.58	0.06	8
Early maturity	0.63	0.00	5
Chenopodium album			
Young	3.78	0.04	.....
Just before blooming	3.50	0.16	70
Early maturity	0.92	Trace	8
Salsola Kali			
Young	2.66	Trace	.....
Just before blooming	3.16	0.06	3
Early maturity	1.19	0.00	2
Amaranthus blitoides			
Young	3.50	0.10	.....
Just before blooming	3.69	0.27	34
Early maturity	2.10	Trace	24
Amaranthus graecizans			
Young	4.31	0.15	.....
Just before blooming	4.00	0.50	26
Early maturity	1.01	Trace	22
Portulaca oleracea			
Young	4.48	0.26	.....
Just before blooming	2.85	0.12	26
Early maturity	1.51	Trace	7

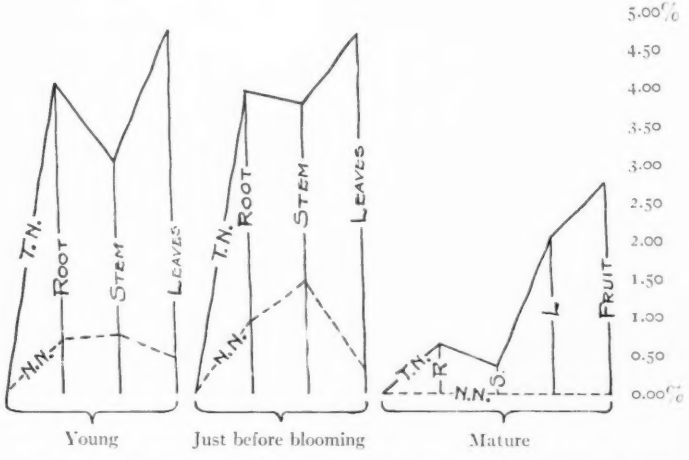

FIG. 2.—Ratio between total nitrogen (T.N.) and nitrate nitrogen (N.N.) in *Amaranthus retroflexus* in three stages of growth, taken under field conditions.

progressively lower from young to mature stages. *Capsella Bursa-pastoris* leads all other species in this series in the first two stages of growth, both in total and nitrate nitrogen.

Soil samples were taken at the roots of seven species just before blooming and at maturity, and tested for nitrate nitrogen, which was calculated in pounds per acre (2), as shown in table VI.

The third growth stage of the plants used in table VI is early rather than full maturity, as in all other cases. In this stage a

TABLE VII

Stage	Part	Percentage total nitrogen	Percentage nitrate nitrogen	Lb. of nitrate in soil per acre
Atriplex patula (on well decayed hog hair)				
Young	.....	3.46	0.22	.....
Just before blooming	.....	3.70	0.46	60
Early maturity	.....	1.87	0.12	9
Amaranthus retroflexus (on decayed hog hair)				
Young	.....	4.50	0.65	.....
Just before blooming	.....	4.55	0.52	140
Early maturity	.....	1.47	0.08	75
Amaranthus retroflexus (in edge of horse stable)				
Young	.....	4.97	0.36	.....
Just before blooming	.....	4.62	0.56	650
Early maturity	.....	2.60	0.25	1175
Amaranthus retroflexus (on manure pile)				
Young	Root	2.21	Trace	.....
	Stem	2.42	Trace	.....
	Leaves	4.06	0.00	.....
Just before blooming	Root	2.70	0.38	.....
	Stem	3.47	0.56	.....
	Leaves	4.29	0.10	.....
Mature	Root	1.59	0.40	.....
	Stem	1.41	0.45	.....
	Leaves	3.75	Trace	.....
	Fruit	3.92	Trace	.....

trace of nitrate was usually found, whereas at full maturity none was found.

By neither method employed was nitrate nitrogen found in a fully matured specimen of *Amaranthus retroflexus*, grown under field conditions, nor in any of the other twenty-four species tested. The nitrate curve rises gradually from the early seedling stage to the blooming stage, where it begins its decline, reaching zero at full maturity (fig. 2). Nearly all the annuals tested in early maturity

showed at least a trace of nitrate. This regular nitrate curve was not obtained in the case of plants grown under abnormal conditions. Table VII gives the results obtained on *A. retroflexus* for two successive years, in part, and *Atriplex patula* for one year. In these specimens the nitrate was not reduced to a trace at early maturity, nor was the zero mark reached at full maturity.

TABLE VIII

Plant	Season	Percentage total nitrogen	Percentage nitrate nitrogen
Capsella Bursa-pastoris	Spring	4.62	0.15
	Fall	4.20	0.12
Erigeron annuus	Spring	3.36	0.12
	Fall	2.10	0.04
Lactuca scariola	Spring	3.36	0.08
	Fall	3.60	0.10
Potentilla monspeliensis	Spring	3.36	0.12
	Fall	3.50	0.08
Daucus carota	Spring	3.68	0.12
	Fall	2.29	0.04
Arctium minus	Spring	2.78	0.13
	Fall	2.15	0.08
Rumex crispus	Spring	3.22	0.10
	Fall	2.45	0.04
Solanum carolinense	Spring	3.12	0.12
	Fall	1.75	0.00
Cirsium arvense	Spring	4.27	0.20
	Fall	1.68	0.10
Asclepias syriaca	Spring	.....	.....
	Fall	1.35	0.00
Physalis heterophylla	Spring	.....	.....
	Fall	1.82	0.00

Specimens of certain species of winter annuals and biennials were collected in the fall, and compared as to nitrogen content with specimens of the same species taken in the spring. It was found for most species that the percentages were higher in the spring (table VIII).

Those perennials tested whose above-ground portions mature and die at or before the first frost contain no nitrate at the beginning of winter, namely, *Solanum carolinense*, *Asclepias syriaca*, and *Physalis heterophylla*. The perennials whose lower leaves remain green after frost, however, as *Cirsium arvense*, *Rumex crispus*, etc.,

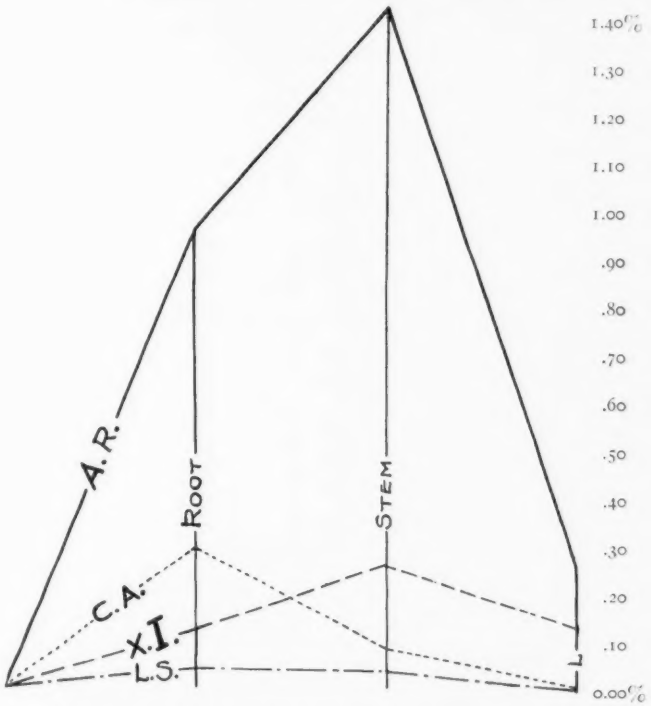

FIG. 3.—Percentage relations of nitrate nitrogen in *Amaranthus retroflexus* (A.R.), *Xanthium italicum* (X.I.), *Chenopodium album* (C.A.), and *Lactuca scariola* (L.S.), taken under field conditions just before blooming.

not only have some nitrate nitrogen in early winter, but contain a larger portion of total nitrogen than do those perennial species whose aerial portions die at frost. It has been suggested that the ability of *Amaranthus retroflexus* to absorb great quantities of nitrates might be an inherited trait, and that other species of the same and

possibly the preceding and succeeding alliances may have the same tendencies.

In the analyses of 1920 and 1921 only four species showed any appreciable and consistent amount of nitrate nitrogen, namely, *Amaranthus retroflexus*, *Chenopodium album*, *Xanthium italicum*,

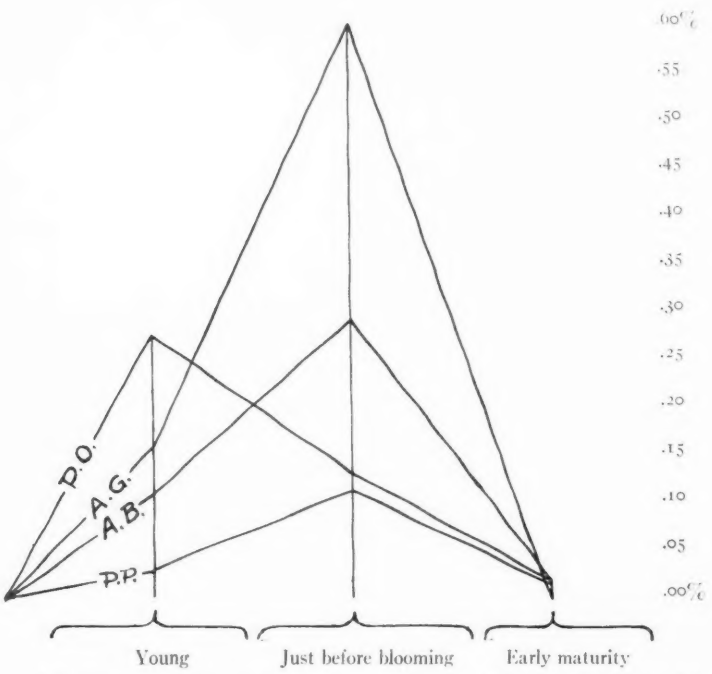

FIG. 4.—Nitrate nitrogen in *Amaranthus graecizans* (A.G.), *A. blitoides* (A.B.), *Portulaca oleracea* (P.O.), and *Polygonum pennsylvanicum* (P.P.), taken under field conditions in three stages of development.

and *Lactuca scariola*. The nitrate ratio of these species is shown in fig. 3. Of the additional species tested in 1922, it was found that the four plants leading in nitrate nitrogen content involved only Chenopodiales and its two adjacent alliances, Polygonales and Caryophyllales, with Chenopodiales again leading. Table VI shows the analyses of species ranging through Polygonales, Cheno-

podiales, and Caryophyllales. Chenopodiales, the alliance in which the pigweeds are found, leads the preceding and succeeding alliances in nitrate content. Figures taken from the various tables of this paper, representing plant alliances ranging from Graminales to Campanales, indicate a slight but consistent lead for Chenopodiales in regard to nitrate nitrogen absorption. Fig. 4 shows the percentage relations of nitrate in *Amaranthus graecizans*, *A. blitoides*, *Portulaca oleracea*, and *Polygonum pennsylvanicum*. Here again *Amaranthus* is a strong leader. Of the twenty-one genera involved in the analyses of this paper, under no conditions has *Amaranthus* been surpassed. In plants growing under normal conditions, the largest amount of nitrate nitrogen was invariably found in that stage of growth just before blooming, the lowest percentage in early maturity, zero being reached at full maturity.

Summary

1. Nitrate nitrogen, prominent in young plants of *Amaranthus retroflexus* and some other weeds, during young and immature stages, completely disappears at full maturity, contrary to Woo's prediction. Twenty-five species of weeds have been tested, and none shows nitrate nitrogen in the mature stage.

2. In all species grown under normal conditions, the highest percentage of nitrate is found in that stage of development designated as just before blooming.

3. *Amaranthus retroflexus* and *Atriplex patula*, grown upon manure piles and heaps of decaying hog hair, vary from what seems to be the normal course in nitrate absorption. In these cases early maturity shows more than a trace, and at full maturity nitrate is still present.

4. Of the two methods followed in nitrate determination, the colorimetric was found to have advantages over the volumetric, in its ease of operation and certainty of results.

The writer is grateful to Dr. William Crocker, Dr. Charles A. Shull, Dr. R. H. Carr, and Mr. P. H. Brewer for valuable suggestions.

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CORRELATION OF FOSSIL FLORAS OF RHODE ISLAND AND NEW BRUNSWICK[1]

EDA M. ROUND

(WITH FOUR FIGURES)

The geographical positions of Rhode Island and New Brunswick make a comparative study of their fossil plants desirable. Such study is facilitated by recent work on the Fern Ledges flora by STOPES (5), in which about forty species from St. John, New Brunswick, have been described. Several of these may be cited as

TABLE I

SPECIES	LOCALITIES IN NARRAGANSETT BASIN												
	Pawtucket	Central Falls	Valley Falls	Lonsdale	Cranston	Arlington	Tunnel, Providence	Silver Spring	Bristol	Portsmouth	Newport	Mansfield, Mass.	Perrins, Mass.
1. Calamites sp.	...	*	...	...	...	...	...	...	...	...	...	...	...
2. Calamites Suckowi	*	...	*	...	...	...	...	*	...	...	...	...	...
3. Annularia sphenophylloides	*	...	*	*	*	*	...	*	...	...	*	*	*
4. Annularia stellata	*	...	*	...	*	...	...	...	*	*	*	*	...
5. Sphenopteris valida	...	...	*	...	...	...	...	...	...	...	...	...	...
6. Oligocarpia splendens	...	...	*	...	...	...	...	...	...	*	...	...	...
7. Pecopteris Miltoni	*	...	*	...	...	...	...	...	...	...	...	...	...
8. Pecopteris plumosa	*	...	...	...	...	...	...	*	...	...	...	...	...
9. Neuropteris heterophylla	*	...	*	*	*	...	*	...	*	*	...	...	...
10. Cordaites cf. Robbii	*	...	...	...	...	...	...	...	...	...	...	...	...

belonging to the Rhode Island coal. They include some of the most common and widespread species in the state, the names and localities of which are given in table I.

If the species listed are considered separately, certain facts become apparent.

1. *Calamites* sp. is a closely striated specimen with the same general size and internodal distance characteristic of the Canadian form. It is represented in Rhode Island both as fossil and cast, but, being indeterminable, is of slight correlative value.

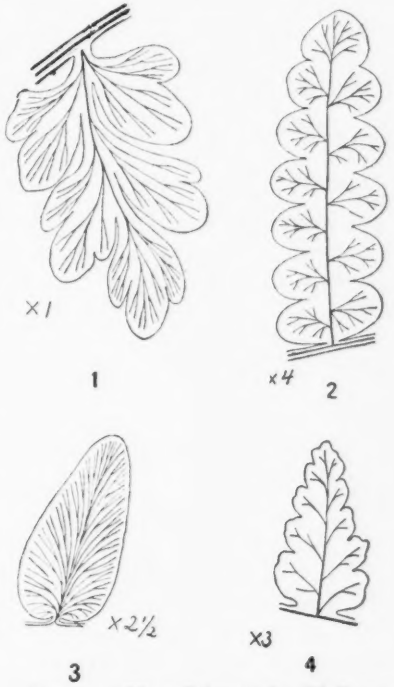
[1] Work started in partial fulfilment of requirement for the degree of Ph.D. at Brown University.

2. *Calamites Suckowi* Brgt. has been reported from all parts of the Upper Carboniferous in North America, although most characteristic of the Allegheny and Conemaugh formations. These horizons may be designated as Upper Westphalian and Lower Stephanian respectively, where correlation with Europe is desired (4).

3. *Annularia sphenophylloides* Gutbier, while appearing in all horizons of the Pennsylvanian period, seems to have been most widespread during the Conemaugh and Monongahela divisions thereof.

4. *Annularia stellata* Wood appears to have been most characteristic of the Allegheny and Conemaugh. Although it is common in the Cherokee shales of Missouri, a horizon regarded as lower productive coal (6), JONGMANS (2) regards it as typical of the upper part of the Upper Carboniferous, an opinion based largely on European conditions.

5. *Sphenopteris valida* Daws. (fig. 1) is regarded in Europe as a characteristic Westphalian species (5).



FIGS. 1–4.—Fig. 1, *Sphenopteris valida* Daws.: detail showing veining; drawn from specimen no. 868, Brown University coll.: fig. 2, *Pecopteris Miltoni* Artis: enlarged detail, ×4; drawn from specimen no. 157, Brown University coll.: fig. 3, *Neuropteris heterophylla* Brongn.: enlarged detail, ×2.5; drawn from specimen no. 354, Brown University coll.: fig. 4, *Oligocarpia splendens* Daws. sp.: enlarged detail, ×3; drawn from specimen no. 315, Brown University coll.

6. *Oligocarpia splendens* Daws. (fig. 4) is common to the Valenciennes flora of France, which is called Westphalian in horizon (7).

7. *Pecopteris Miltoni* Brgt. (fig. 2) appears to have been abundant in the lower coal of Europe (1), and so is useful as an index fossil of that period.

8. *Pecopteris plumosa* Artis sp. is occasionally found in the Narragansett Basin, being apparently a narrow, simple form of *Pecopteris dentata* Brgt. STOPES states that "the species is widely distributed in the Wesphalian and is particularly common in the Middle Coal Measures of Britain" (5).

9. *Neuropteris heterophylla* Brgt. (fig. 3), which is very common in the principal fossiliferous sections of Rhode Island, is interesting to botanists because of the fact that seeds have been found attached thereto (3). This characteristic shows the plant to have been a pteridosperm. It is the first supposedly fern type known to have produced seeds, thus furnishing an evolutionary link between the pteridophytes of the Carboniferous and modern flowering plants. While its vertical range extends from the Pottsville to the Dunkard formations, *Neuropteris heterophylla* is most characteristic of the Westphalian series in Europe.

10. *Cordaites* cf. *Robbii* Daws. is regarded as probably synonymous with *Cordaites borassifolius* Sternb., a common Rhode Island species. This genus represents the only group of gymnosperms from the coal shales of the Narragansett Basin.

While the species of fossil plants common to Fern Ledges and Rhode Island at present are too few for detailed correlation, the similarities between the localities are worthy of consideration, and should be helpful in making knowledge of the Carboniferous formations of Rhode Island more complete.

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LITERATURE CITED

1. HOFMAN, A., and RYBA, F., Verbreit. der pal. Leitpf. in den mitteleu. Kohlenabl. 1899.
2. JONGMANS, W. J., Anleit. z. Bestim. d. Karbonpf. W. Europas. 1911 (p. 250).
3. KIDSTON, R., Phil. Trans. B. **197**:1:5. 1904.
4. KNOWLTON, F. H., Evolution of geologic climates. Bull. G.S.A. **30**:512. 1919.
5. STOPES, M. C., G.S.C. Geol. Ser. Mem. no. 38, 41. 1914.
6. UNITED STATES GEOLOGICAL SURVEY MONOGRAPH 37. p. 161. 1899.
7. ZEILLER, R., Flore fossile Valenciennes. 1888 (p. 100).

THREE NEW SPECIES OF BENGAL POLYPORACEAE

S. R. Bose

(WITH THREE FIGURES)

Polyporus Guhae, n.sp.—Pileus almost sessile or with short stalk, about 2 mm. thick, very hard, somewhat fan-shaped or petaloid, 2–4 × 2.5–3 cm.: upper surface smooth, dull or dark brown, distinctly zoned, with raised ridges: hymenial surface blackish, faint yellow (with a brown tinge), shining; pores round, very minute, pore tubes longer than the context, which is very thin: margin with a black line, slightly involute: setae none: spores not found.

FIG. 1.—*Polyporus Guhae*, upper and lower surfaces.

In imbricate clusters on rotten wood amongst grass, in interior of Howrah District, July 1916.

This species belongs to section Petaloides 15 of LLOYD'S paper on "Stipitate Polyporoids" (thin, rigid Polypores, pale rose or reddish brown). LLOYD remarks: "We would enter this in section 15 of our pamphlet; on account of its rigidity and hardness it might be sought in *Trametes*, but the different color of pores and the context forbid."

Trametes Karii, sp. nov.—Pileus almost sessile, with short lateral stalk, somewhat reniform, soft, becoming somewhat coriaceous in drying, 7 × 4.5 cm.; also some very small forms, about 6 mm. thick, pale internally: upper surface glabrous, yellowish brown, a few faint rings toward the margin: hymenial surface dark brown; pores small, mostly round, pore tubes 3–4 mm. deep: margin entire, with a thin black line: setae none: spores not found.

In imbricate groups of three or four on dead wood amongst grass, Darjeeling, July 1918; Coimbatore, Madras, September 1921. Named for the late Dr. R. G. KAR, one of the founders of the Carmichael Medical College.

LLOYD remarks: "The plant impresses me as probably a white plant in its prime, being spotted brown when old or in drying, particularly when bruised. I should put it in section 128 of my journal, although it suggests the white plants in section 124."

FIG. 2.—*Trametes Karii*, upper and lower surfaces

FIG. 3.—*Favolus Bengala*, upper and lower surfaces

Favolus Bengala, sp. nov.—Pileus almost sessile or with short lateral stock, about 2 mm. thick, coriaceous, 2–4 cm. long, 2–3 cm. broad, tapering toward the base, expanded at the margin: upper

surface perfectly smooth, dull white, marked by faint concentric rings: hymenial surface with pores fairly large, elongated, somewhat irregular and crenate; pore mouths yellowish, pore tubes deep, 2–5 mm. long, context very small: marginal outline wavy, sometimes broken up into corners: setae none: spores very few and rare, globose, 4 μ in diameter, almost hyaline.

On dead wood, in imbricate groups, interior of Hugli District, Bengal, August 1916.

LLOYD remarks: "This is an ambiguous *Favolus*, but we think it should be so classed on account of its large elongated pores, which are arranged in lines, indicating its affinities to a lamelliform arrangement. The rigid, firm texture of the plant, however, suggests *Trametes*."

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BRIEFER ARTICLES

A METHOD OF SECURING SPORES OF YEAST

One of the methods generally recommended for obtaining endospores of yeast is to grow the yeast on sterile blocks of plaster of Paris, a method perfected by HANSEN[1] a number of years ago. During the spring of 1923 the writer discovered that the surface layers of cakes of Fleischmann's yeast which had been kept in an ice chest in the laboratory for several (6–8) days contained an abundance of endospores. No spores were present in the interior of the cakes, but if the cakes were broken open spores developed on the broken surfaces in a few days, either in the ice chest or at room temperature.

At various times later, four more lots of Fleischmann's yeast (3 cakes in each case) were examined with similar results. Sometimes there were many spores present in the surface layers of the cakes as soon as they were received. Spores, however, always developed or became more abundant on keeping the cakes a few days.

The spores, which are quite refractive, may be observed fairly well by mounting them in a drop of Gram's iodine and examining under the microscope, using a magnification of about 600 diameters. Very satisfactory stained preparations may be made as follows: (1) spread a film in a small drop of water on a slide and dry in the air; (2) fix by passing through a flame 12 to 15 times; (3) stain with hot carbol-fuchsin 1–3 minutes; (4) wash with water; (5) destain with 5 per cent sulphuric acid 2–3 seconds; (6) wash with water; (7) stain with methylene blue about 3 seconds; (8) wash with water.

Cover and examine under the microscope. The spores should be stained a bright red and the vegetative cells or parts of cells a shade of blue. The wall stains only slightly, but may be seen satisfactorily if the mount is examined in water. If the cells are cleared in the usual way, and mounted in balsam, the wall of the ascus becomes invisible. The stained mounts are permanent if allowed to dry in the air, however, and may be examined at any time by covering with a drop of water and a cover glass. They may also be examined with an immersion objective

[1] TANNER, F. W., The Yeasts. Translation. 1920 (p. 152).

by applying immersion oil without a cover glass directly to the dry mount, but this renders the wall practically invisible.

Pure cultures may readily be obtained by the poured plate method, using potato dextrose agar (2 per cent dextrose) or a similar sugar medium. In such cultures some spores will develop in 4–6 days, especially on the upper parts of agar slopes. A much greater abundance of spores may be obtained, however, if transfers of the yeast are made from pure cultures to an agar medium prepared according to the following formula: distilled water, 100 cc.; Liebig's beef extract, 0.3 gm.; sodium chloride, 0.5 gm.; dextrose, 0.25 gm.; agar 1.5 to 2 gm. Sterilize at 12 or 15 pounds pressure.

On this medium,[1] which is a modification of that used by GORODKOWA, very good spore production resulted in 4–5 days, and in 8–12 days practically 50 per cent of all cells developed spores.

Cakes of "yeast foam" have also been examined, but no spores were found in them. When isolations from such cakes were made and the yeast grown on the medium given, spores developed apparently as readily as in cultures from a cake of Fleischmann's yeast.—W. E. MANEVAL, *University of Missouri, Columbia, Mo.*

[1] *Ibid.* p. 151.

CURRENT LITERATURE

NOTES FOR STUDENTS

So-called bars or rims of Sanio.—Since the publication in 1910 of Miss GERRY'S[1] stimulating paper upon their distribution in the Coniferae, the so-called rims or bars of Sanio have received considerable attention in discussions concerning the affinities of fossilized coniferous woods, and in controversies concerning the relative antiquity of the Abieteae and Araucarieae. The conclusions of different investigators are extremely contradictory, not only as regards the nomenclature, form, and chemical composition of these structures, but particularly as regards their distribution in putative "conservative" regions or organs of the Coniferae.

In 1919 the writer[2] pointed out that unconformity between the primary and secondary membranes, and structures resembling the so-called bars or rims of Sanio, tend to appear at times in the Pteridophyta and Angiospermae, apparently as concomitants of processes of modification, especially reduction, in tracheary pitting. Although the writer suggested that the transverse thickenings in *Ginkgo*, *Pinus*, *Larix*, and other Coniferae may be vestiges of an ancestral scalariform structure, rather than modifications of curved rims or structures *sui generis*, he strongly emphasized the fact that until more is known concerning their developmental history, during differentiation from the meristems, the exact significance of the various types of primary pit areas and thickenings of the middle lamella must remain more or less obscure; therefore, their arrangement in phylogenetic sequences is purely conjectural.

SIFTON[3] and HALE,[4] in continuing the controversy concerning the relative antiquity of the Abieteae and Araucarieae, focus their attention solely upon speculations concerning the significance of end products. The suggestion that transverse bars in Coniferae may be vestiges of a scalariform structure is divorced from its context in the writer's paper, and criticized without reference to the emphasis placed upon the necessity for detailed developmental investigations. SIFTON, in supporting THOMSON'S view of the primitiveness of the araucarians, cites the occurrence of bars of Sanio in the secondary wood and conservative regions of cycads as invalidating JEFFREY'S use of similar con-

[1] GERRY, ELOISE, The distribution of the "bars of Sanio" in the Coniferales. Ann. Botany **24**:119–123. 1910.

[2] BAILEY, I. W., Structure, development, and distribution of so-called rims or bars of Sanio. BOT. GAZ. **67**:449–468. 1919.

[3] SIFTON, H. B., The bar of Sanio and primordial pit in gymnosperms. Trans. Roy. Soc. Canada **16**:83–88. 1922.

[4] HALE, J. D., The bars or rims of Sanio. BOT. GAZ. **76**: 241–256. 1923.

servative regions in araucarians. He maintains that bars are formed by vertical fusion between the rims of closely approximated primordial pits, in the higher gymnosperms by horizontal fusion of shorter bars and rims. Rims and primitive bars occur in conservative regions of *Pinus* and *Ginkgo*. On the contrary, HALE asserts that true bars or rims of Sanio characteristically occur in the secondary wood only of the Ginkgoales, Coniferales, Gnetales, and Angiospermae, and are absent in primary elements. The presence of bars in primitive regions of araucarians and their absence in similar regions of *Ginkgo* and *Pinus* is reiterated as evidence for the derivation of the araucarians from pinelike ancestors.

Although HALE errs in stating that unconformity does not occur in the tracheids of ferns and cycads, most discrepancies concerning the presence or absence of the so-called bars or rims of Sanio in different plants, organs, and tissues are due largely to varying definitions of these structures. Thus HALE coins his own definition of "true" bars or rims of Sanio, and brushes aside SIFTON's figures of bars in the secondary wood of *Dioon* as "not convincingly exemplified." It is significant in this connection, as previously noted by the writer, that the observations of KRÜGER, DEBARY, JANCZEWSKI, RUSSOW, STRASBURGER, DIPPEL, and others upon the occurrence of primary pit areas and thickenings in the walls of the cambium, and their relation to similar structures in the elements of the xylem and phloem, have been overlooked in discussions concerning the so-called rims or bars of Sanio. The writer's recent investigations upon the cambium and its derivative tissues strengthen his contention that extensive developmental investigations are essential if the protracted controversy concerning the significance of so-called bars or rims of Sanio is not to become a *reductio ad absurdum*.—I. W. BAILEY.

Respiration and photosynthesis.—In an important contribution, SPOEHR and MCGEE[5] show that the carbohydrate content alone of leaves is no index of their respiratory activity. They show that the amino acid content of the leaves is very important. Using for most of the experiments excised leaves of *Helianthus annuus* and the "Canada Wonder" bean, they show that the general course of respiration in the case of leaves taken from the plant is downward. This is due to the decreasing carbohydrate content of leaves kept in the dark. If the leaves are fed sugar, this decrease in carbohydrates is much less pronounced. In this case respiration decreases during the first 30–35 hours, when there is an increase to a point higher than the initial rate. By numerous experiments, it is shown that this increase in respiration is due to an accumulation of amino acids in the leaves kept in the dark. The amino acids stimulate respiration, and, as a result, accompanying a decrease in carbohydrate content there is an increase in respiration. Such experiments, as well as experiments

[5] SPOEHR, H. A., and MCGEE, J. M., Studies in plant respiration and photosynthesis. Carnegie Inst. Wash. Publ. 325. pp. iv+98. 1923.

showing a decrease in respiration correlated with an increase in carbohydrate content, show that the carbohydrate content alone cannot be used as a measure of respiration. When leaves were fed amino acids a stimulating effect on respiration was noted, especially when there was also sugar in the nutrient solution. Of the sugars used, d-glucose seemed to cause the greatest activity in respiration, d-levulose the least, while sucrose was midway between the two; d-mannose had much the same effect as d-glucose. Also, amino acids used in connection with d-levulose did not stimulate respiration, while the effect was marked in the case of d-glucose and d-mannose. The experiments give no explanation of this effect of amino acids, but, in the literature discussion, the effect of amino acids in stimulating enzyme activity is emphasized.

A few experiments are recorded showing a relationship between respiration and photosynthesis. If conditions were such that respiratory activity was low in the leaves, photosynthesis was also weak; and a high respiratory rate was associated with a high photosynthetic rate. SPOEHR prefers to let further experiments explain this relationship. In the introductory discussion some speculations are put forward, and the suggestion made that in this relationship we may possibly have an explanation of the internal factor of photosynthesis (WILLSTATTER'S enzymatic reaction).

The literature citations are numerous, and the apparatus and methods are such as to insure the highest accuracy, and also to enable a large number of determinations to be made in a short time.—S. V. EATON.

Forest ecology.—The quantitative study of the factors of environment represents one of the most fruitful lines of investigation in modern ecology, and it is an indication of progress when foresters apply such methods to the solution of their problems. In a recent contribution, BATES[6] tells of the collection of such quantitative data for climatic and soil conditions at the Fremont Experiment Station, Manitou, Colorado. These are to be summarized in a future publication, the present one dealing with the equally important physiological requirements of the principal tree members of the Colorado forests The species receiving most attention are *Pinus ponderosa*, *Pseudotsuga Douglasii*, *P. Murrayana*, and *Picea Engelmannii*. These are compared as to their water requirement, sap density, non-available soil moisture, and resistance to high temperature and to winter drought. In the efficient use of both water and sunlight, as indicated by water requirement and sap density caused by abundance of carbohydrates, the spruce stands first, followed by the Douglas fir, lodgepole, and yellow pine in the order named. In the economy of water per unit leaf area exposed the order remains the same, except that the positions of yellow and lodgepole pine are reversed. This latter arrangement also expresses the resistance to winter killing. The economy in the use of both

[6] BATES, C. G., Physiological requirements of Rocky Mountain trees. Jour. Agric. Res. 24:97–164. 1923.

water and sunlight energy on the part of the Engelmann spruce may explain to a considerable extent its shade tolerance and its success in competition.

Considerable attention is devoted to the relation of the trees to soil moisture, and it is interesting to note that the seedlings of the spruce, Douglas fir, and the yellow pine have essentially the same wilting coefficient, while the lodgepole pine has one somewhat in excess of the others. Here, as in the other studies, only seedlings are used, and BATES recognized that they may possess internal characters somewhat different from mature individuals, but, as he points out, it is the performance of seedlings rather than that of older trees which determines the composition of forest stands.

Too much emphasis can hardly be placed upon the importance of studies of this sort, especially when accompanied, as in this instance, with others of environmental factors. The task of correlating the two will not be a light one, but it promises to be most fruitful in leading to a much better understanding of the problems of forest development, whether under natural or under the partially controlled conditions of forestry.—GEO. D. FULLER.

A genetic study of Splachnum.—In addition to describing the morphology and anatomy of *Splachnum sphaericum* as it appears in nature and in cultures, SCHWEIZER[7] reports the results of experiments with this moss suggested by the well known earlier work of É. and É. MARCHAL. The species proves to be strictly dioecious; that is, all the gametophores produced by a given protonema bear only antheridia or only archegonia. Secondary protonemata from any portion of a gametophyte produce gametophores of the same sex as that of the parental plant. From wounded portions of immature sporophytes protonemata were obtained which gave rise to (supposedly) diploid gametophores. Most of the diploid gametophytes, including protonemata and gametophores, closely resembled the haploid gametophytes; although numerous measurements showed differences in the average sizes of cells of certain types, the difference in each case being in favor of the diploid gametophyte. A considerable proportion of the diploid gametophytic clones were bisexual in the sense that each produced gametophores bearing antheridia and others bearing archegonia, but no bisexual gametophores appeared. Some diploid clones, however, so far as observation disclosed, produced only female gametophores. Three diploid clones constantly showed various marked aberrancies; two of these "monstrous" clones were strictly female, the third was strictly male. The eggs borne by the "normal" diploid gametophytes and by one of the "monstrous" forms were readily fertilized. This condition differs from that in the dioecious mosses investigated by the MARCHALS, the eggs of which proved incapable of fertilization. By the union of diploid antherozoids with diploid eggs, tetraploid sporophytes were obtained. Spores from these sporophytes gave rise to

[7] SCHWEIZER, J., Polyploidie und Geschlechterverteilung bei *Splachnum sphaericum* (Linn. fil.) Swartz. Flora **116**:1–72. 1923.

(presumably) diploid gametophytes, which to the time of writing had proved unisexual, four bearing only antheridia, three only archegonia. Wounded tetraploid sporophytes gave rise by means of protonemata to tetraploid gametophytes, which, so far as reported, had borne antheridia but no archegonia.—C. E. ALLEN.

Gametophyte and embryo of Botrychium simplex.—CAMPBELL[8] has investigated *Botrychium simplex*, the smallest species of the genus, and related to *B. Lunaria*. The gametophyte closely resembles that of *B. Lunaria* in size and structure, but is much smaller than that of the other species examined. As in other species of the genus, the presence of a ventral canal cell is doubtful. More than one archegonium may be fertilized, but only one sporophyte was observed to develop from the same gametophyte. The embryo also resembles that of *B. Lunaria*, but the early divisions are variable and much more irregular. Details of embryo development are given and contrasted with other species. The vascular "skeleton" of the young sporophyte is said to be made up exclusively of the steles belonging to the roots and leaves.—J. M. C.

Protoplasmic streaming.—To make the demonstration of protoplasmic streaming easier, SEIFRIZ[9] has studied the influence of various reagents on streaming in leaves of *Elodea*. He finds that methyl alcohol, the glucosides, saponin, senegin, smilacin, and strontium and barium chlorides, in dilute solutions of about two to five per cent, cause abnormal streaming after immersion for about two or three days. Copper in such concentrations as might occur in distilled water from a copper still also induces streaming. The types of streaming are described and suggestions as to possible causes of the stimulation are made.—W. B. DAVIS.

Embryogeny of Solanaceae.—SOUÈGES,[10] in a series of four papers, presents the results of his investigations of the embryogeny of representative Solanaceae, the genera used being *Nicotiana*, *Hyoscyamus*, *Datura*, *Atropa*, and *Solanum*. A general summary organizes the situations that characterize the family.—J. M. C.

North American Flora.—The third part of volume 24 is a continuation of the Rosales by AXEL RYDBERG, including two tribes of the Fabaceae (Indigofereae and Galegeae in part). *Indigofera* is represented by 50 species, 11 of which are new. Of the tribe Galegeae six genera are presented, much the largest being *Cracca* (72 species, 16 new) and *Brongniartia* (37 species, 9 new).—J. M. C.

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[8] CAMPBELL, D. H., The gametophyte and embryo of *Botrychium simplex*. Ann. Botany **36**:441–455. *pl. 16. figs. 10.* 1922.

[9] SEIFRIZ, WM., A method for inducing protoplasmic streaming. New Phytol. **21**:107–112. 1922.

[10] SOUÈGES, R., Recherches sur l'embryogénie des Solanacées. Bull. Soc. Bot. France **69**:163–178, 236–241, 352–365, 555–585. *figs. 259.* 1922.